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Chiral derivatives of xanthones: synthesis, enantiomeric purity and effect on human tumor cell lines

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"Quando penso que cheguei ao meu limite,
descubro que tenho forças para ir além"
Ayrton Senna

Abstract

In recent years, the relationship between chirality and biological activity has been of increasing importance in Pharmaceutical Medicinal Chemistry. Actually, chirality can now be considered as one of the majors topics in the design, discovery, development and marketing of new drugs. Considering a particular biological activity we find frequently that one of the enantiomers is more potent than the other. The differences normally exhibited by the enantiomers in pharmacodynamics, pharmacokinetics and toxicity, makes the therapeutics with single enantiomers, comparing with racemates, of unquestionable advantages.

Xanthones (9*H*-xanthon-9-ones) are privileged structures with a large range of biological and pharmacological activities. Among them, carboxyxanthone derivatives (XCars) are considered not only interesting bioactive compounds but they are also very important chemical substrates to obtain new compounds, such as chiral derivatives. Even though there is a large structural multiplicity of bioactive xanthonic compounds, there are only a few reports in literature about synthetic chiral derivatives of xanthones (CDXs). Moreover, CDXs represent an attractive area of chemical research, since they exhibit important biological/pharmacological activities. In some cases the biological activities are dependent on the stereochemistry of the CDXs.

This dissertation reports the synthesis of new CDXs in enantiomerically pure form and the evaluation of their growth inhibitory effect on three human tumor cell lines. The evaluation of the enantiomeric purity of the synthesized CDXs by enantioselective liquid chromatography using (*S*,*S*)-Whelk-O1 chiral stationary phase was also considered. Three XCars were also successfully synthesized by a multi-step synthetic pathway.

The CDXs were synthesized by coupling two XCars as chemical substrates with a variety of commercially available enantiomerically pure building blocks, using the reagent *O*-(benzotriazol-1-yl)-*N*-*N*-*N*'-*N*'-tetramethyluronium tetrafluoroborate (TBTU), with short reactions times (20 min to 1.5 h), excellent yields (from 95 to 99%) and very high enantiomeric purity (enantiomeric excess higher than 99%).

Considering biological activity evaluation, some CDXs exhibited interesting results with enantioselectivity. Furthermore, the nature and positions of substituents on the xanthonic scaffold of CDXs demonstrate to have great influence on the growth inhibitory effect.

Keywords: Chirality; Enantiomeric Purity; Single enantiomer; Chiral derivatives of xanthones; Enantioselectivity.

Resumo

Nos últimos anos, a relação entre quiralidade e atividade biológica tem sido cada vez mais importante na área farmacêutica, com especial destaque para a Química Farmacêutica Medicinal. Atualmente, a quiralidade pode ser considerada como um dos grandes temas, no *design*, descoberta, desenvolvimento e comercialização de novos fármacos. Considerando as atividades biológicas frequentemente se constata que um dos enantiómeros é mais potente do que outro. As diferenças normalmente exibidas pelos enantiómeros, quer na farmacodinâmica, farmacocinética e toxicidade, reforçam a importância da utilização dos enantiómeros puros comparativamente com os racematos.

Os derivados xantónicos são considerados estruturas privilegiadas com uma grande variedade de atividades biológicas e farmacológicas. Além disso, os derivados xantónicos carboxilados (XCars) são considerados não apenas interessantes compostos bioativos, mas também são substratos químicos muito importantes para a obtenção de novos compostos, como derivados xantónicos quirais (DXQs). Apesar da grande multiplicidade estrutural de compostos xantónicos bioativos, estão apenas descritos alguns exemplos na literatura sobre DXQs. Estes representam uma área de investigação muito apelativa, uma vez que estudos descritos têm demonstrado que exibem actividades biológicas/farmacológicas importantes. Além disso, em alguns casos as atividades biológicas são dependentes da estereoquímica do DXQ.

Esta dissertação descreve a síntese de novos DXQs na forma enantiomericamente pura e a avaliação do efeito inibidor do crescimento de três linhas celulares tumorais humanas. Também foi considerada a avaliação da pureza enantiomérica dos DXQs sintetizados através de cromatografia líquida enantiosseletiva, utilizando a fase estacionária quiral (*S*,*S*)-Whelk-O1. Foram também sintetizadps com sucesso três XCars por uma via de síntese em múltiplos passos.

Os DXQs foram sintetizados por acoplamento de dois XCars como substratos químicos com uma grande variedade de blocos moleculares enantiomericamente puros, disponíveis comercialmente, utilizando o reagente tetrafluoroborato de *O*-(benzotriazol-1-il)-*N*-*N*-*N*'-*N*'-tetrametilurónio (TBTU), com tempos reacionais curtos (20 min a 1,5 h), rendimentos excelentes (95 a 99%) e excesso enantiomerico normalmente maior que 99%.

Considerando a avaliação da atividade biológica, alguns DXQs apresentaram resultados interessantes de enantioseletividade. Além disso, a natureza e as posições dos substituintes no núcleo xantónico demonstraram ter influência sobre o efeito de inibição do crescimento celular.

Tanto quanto é do nosso conhecimento os vinte e quatro novos DXQs e um XCar são descritos pela primeira vez nesse trabalho.

Palavra-chave: Quiralidade; Pureza enantiomérica; Enantiómeros puros; Derivados xantónicos quirais; Enantioseletividade.

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Abbreviations and Symbols

$[\alpha]_D$	Specific rotation
α	Rotation
δ	Chemical shift (ppm)
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
AA	Sodium arachidonate
ACN	Acetonitrile
ADMET	Absorption, distribution, metabolism, excretion and toxicity
ADP	Adenosine-5'-diphosphate
$CDCl_3$	Deuterated chloroform
CDX	Chiral derivative of xanthone
CNS	Central nervous system
CPMP	Committee for Proprietary Medical Products
CSP	Chiral stationary phase
d	Doublet
dd	Double doublet
ddd	Double double doublet
DMXAA	Dimethylxanthenone-4-acetic acid
DMSO	Dimethyl sulfoxide
DMSO-d_6	Hexadeuterodimethyl sulfoxide
DQX	Derivados
e.e.	Enantiomeric excess
FAB-MS	Fast atom bombardment mass spectrometry
FDA	Food and Drug Administration
GC	Gas chromatography
GI_{50}	Concentration of compound that was able to cause 50% cell growth inhibition
GSS	Grover, Shah and Shah reaction
Hex	*n*-Hexane
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid chromatography
HSQC	Heteronuclear Single Quantum Coherence

Hz	Hertz
IR	Infrared Spectroscopy
J	Coupling constant (Hz)
k	Retention factor
LC	Liquid chromatography
LPS	Lipopolysaccharide
m	Multiplet
MeOH	Methanol
MES	Maximal electroshock-induced seizerus
NME	New molecular entity
PHA	Phytohemagglutinin
PKC	Protein kinase C
PMA	Phorbol myristate acetate
Rs	Resolution
r.t.	Room temperature
s	Singlet
SAR	Structure activity relationship
ScMet	Subcutaneous pentylenetetrazol seizures
SRB	Sulforhodamine B
$\mathbf{t_0}$	Dead time
TBTU	*O*-(benzotriazol-1-yl)-*N*-*N*-*N*'-*N*'-tetramethyluronium tetrafluoroborate
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TNF-α	Tumor necrosis factor-α
$\mathbf{t_0}$	Dead time
TOX	Neurological toxicity
$\mathbf{t_R}$	Retention time
$\mathbf{W_h}$	Half-height
XD	Xanthone derivative
XCar	Carboxyxanthone derivative

General and Specific Objectives

The general objective of this dissertation was the synthesis of new bioactive chiral derivatives of xanthones (CDXs) in enantiomerically pure form.

Considering that this dissertation concerns Pharmaceutical Medicinal Chemistry biological evaluation was also considered.

The specific objectives of this dissertation were:

- Total synthesis of suitable functionalized xanthone derivatives as chemical substrates by multi-step pathways;
- Optimizatione of the synthetic procedures using other reaction conditions;
- Synthesis of a library of CDXs in enantiomerically pure form by using efficient, and mild coupling reactions of carboxyxanthone derivatives (XCars) with chiral building blocks;
- Structure elucidation of the CDXs, as well as the xanthonic chemical subtracts and intermediates;
- Evaluation of the enantiomeric purity of CDXs by liquid chromatography using a chiral stationary phase;
- Screening the inhibitory effect of growth of human tumor cell lines in order to evaluate the *in vitro* behaviour for both enantiomers.

Structure and Organization of the Dissertation

The present dissertation is structured in six chapters.

Chapter 1 - Introduction

The first chapter deals with the theoretical background that supports the developed work, and is sub-divided into six subchapters. A brief introduction of chirality, enantioselectivity, trends in development of chiral drugs, enantiomeric purity, xanthones, with emphasis on both natural and synthetic chiral derivatives of xanthones (CDXs) are referred.

Chapter 2 – Results and Discussion

This chapter focuses on the results and discussion of the developed research work, being sub-divided into three subchapters, concerning the synthesis, structure elucidation, determination of enantiomeric purity and biological evaluation of CDXs.

Chapter 3 – Experimental

The third chapter describes the experimental methodologies used in this work related to the synthesis, determination of the enantiomeric purity and inhibition of growth of human tumor cell lines.

Chapter 4 – Conclusions

The fourth chapter focuses on the main conclusions of the developed work, based on the proposed objectives.

Chapter 5 – References

In this chapter, the references cited throughout the dissertation as well as the browsers used are presented.

Chapter 6 – Appendixes

The chapter 6 is composed by two tables: Table 24 comprises the chemical structures of natural and synthetic CDXs and their biological activities described in literature. Table 25 comprises the chemical structures of the synthesized carboxyxanthone derivatives and CDXs

Chapter 1:

Introduction

1. Chirality: basic principles

Molecules are termed chiral when can exist in two enantiomeric forms, which are mirror images of each other and are non-superimposable in three dimensions (**Figure 1**). The words chiral and enantiomer are derived from Greek words *cheir*, meaning “hand" and *enantios* meaning “opposite", respectively.[1]

Usually, enantiomerism is a result of a different configuration of four substituents (atoms or groups of atoms) arising from an atom, normally carbon. Such atom is called a stereogenic center or stereocenter. In addition to carbon, nitrogen, phosphorus, sulphur, selenium and boron can also produce stereogenic centers.[2] Chirality is a property of the entire molecule, whereas a stereocenter is one of the causes of chirality. Although most of the chiral molecules show central chirality, with one or more stereogenic centers, there are also chiral molecules possessing axial or planar chirality.[3]

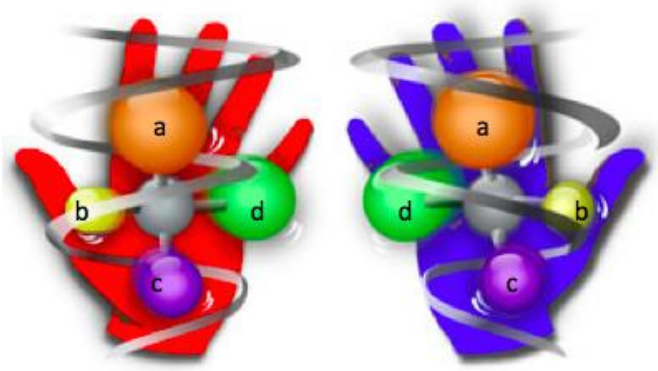


Figure 1: A chiral molecule and its mirror image in a tetrahedral configuration. Letters (a, b, c, and d) represent four different groups attached to a stereogenic center (adapted from[4]).

Configuration can be assigned by the system Cahn-Ingold-Prelog, which expresses the configurations as (*R*) and (*S*) (from the Latin words *rectus* and *sinister* which means right and left, respectively) by a set of priority rules.[5]

Chemically, enantiomers possess the same chemical formula and identical physicochemical properties when they are in an achiral environment, such as density, melting point, boiling point, among others. However, their ability to rotate plane-polarized light is different, and is a property commonly used to identify and characterize two enantiomeric forms: one enantiomer rotates the light to clockwise (dextrorotatory) and the other to counterclockwise (levorotatory), referred by the symbols (+) and (-), respectively. As a consequence, the enantiomers are frequently called optical isomers.[6]

2. Enantioselectivity

It is well know that enantiomers of chiral drugs may possess differences in their pharmacodynamic and pharmacokinetic properties as a consequence of stereoselectivity in drug action and/ or disposition.[6,7] In fact, the biotargets such as receptors and enzymes comprises chiral units being able to recognize and discriminate between enantiomers with high levels of selectivity (**Figure 2**). The enantioselectivity observed in drugs, causes different effects depending on the mode of interaction of the chiral small molecules with the biological macromolecules involved.[8]

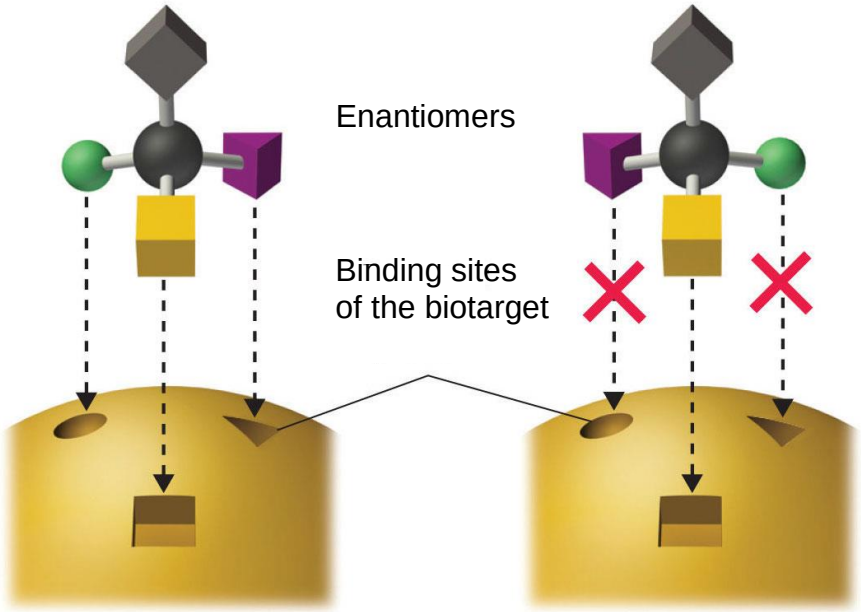
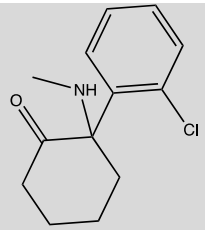
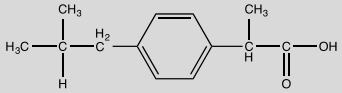
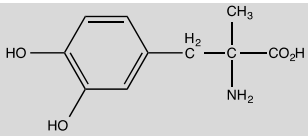
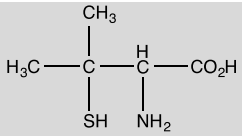
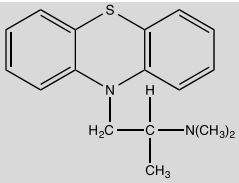
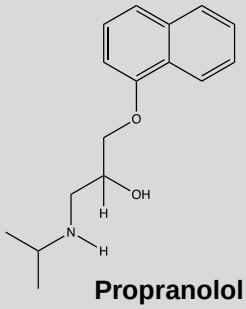
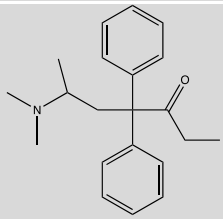


Figure 2: Only one of the two enantiomers shown can achieve three-point binding with the hypothetical binding site of the biotarget (adapted from[9]).

Considering biological activities, when one enantiomer is responsible for the activity of interest, the other enantiomer could be inactive, or possess lower activity, or the some activity, or to be an antagonist or have a different activity that could be desirable or undesirable.[10] Some examples illustrating the referred situations are presented in **Table 1.**[2,11-14]

Regarding a particular biological activity we find frequently that one of the enantiomers is more potent (eutomer) than the other (distomer), being the ratio of the potencies termed eudismic ratio.[15]

Table 1: Examples of different biological activities regarding a pair of enantiomers.

Drug	(*R*)-enantiomer	(*S*)-enantiomer
 **Ketamine**	Hallucinogen	Analgesic Hypnotic
 **Ibuprofen**	Inactive	Antiinflammatory
 **Methyldopa**	Inactive	Antihypertensive
 **Penicillamine**	Mutagenic	Antirheumatic
 **Promethazine**	Antihistaminic	Antihistaminic
 **Propranolol**	Antiarrhythmic	Antiarrhythmic (100X more active)
 **Methadone**	Analgesic 25-50 X more potent	Analgesic

Besides these examples many others demonstrate that enantiomers of chiral drugs should be treated as independent entities rather than just different forms of the same drug.

Considering pharmacokinetics, the distribution, metabolism and excretion processes usually favour one enantiomer over the other.[16] The use of racemic mixtures typically results in stereoselective drug metabolism and may also contribute to the toxicity or adverse effects.[17] Drug absorption is frequently a passive process which depends on properties such as lipophilicity and pKa, and therefore, in this case, the rate extend of absorption usually does not differ for enantiomers.[18] On the other hand, active transport through membranes mediated by a protein transporter may be stereoselective. Regarding the distribution of the drug in plasma, if the plasma protein binding of enantiomers is different at the same total concentration, the active concentration at the site of action will differ.[18] Biotransformation by drug-metabolizing enzymes exhibits the greatest degree of stereoselectivity of all processes contributing to drug disposition.[19] For example, two enantiomers can be metabolized either at different rates or by different routes leading to the preferential metabolism of one enantiomer.[18,19]

Thus, it can be stated that the enantioselectivity plays an essential role in pharmacodynamic, pharmacokinetic and toxicological events. Thus, it is essential to take into account the stereochemistry in drug development to obtain more therapeutic benefits, namely more effective and safer drugs.[20]

Accordingly, there are several advantages in using enantiomeric pure drugs compared to racemates as showed in **Figure 3**.[21]

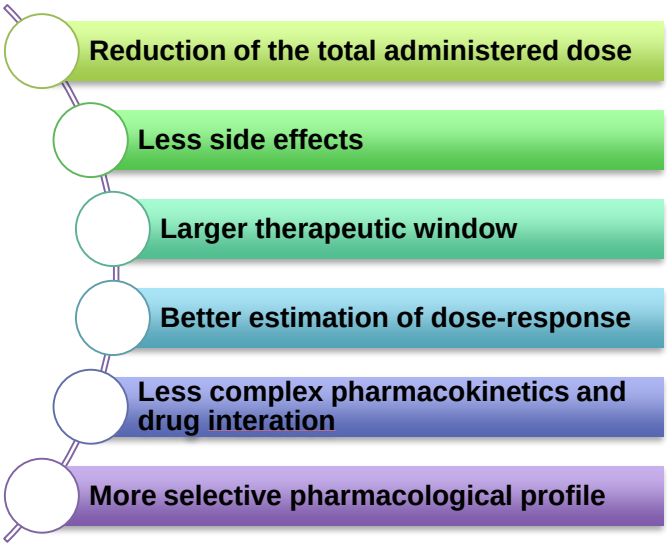


Figure 3: Advantages of enantiomeric pure drugs.

3. Trends in development of chiral drugs

During a large period of time, many of the chiral drugs used in clinical practice were administered as racemates.[10] In 1992, the Food and Drug Administration (FDA)[22] announced a set of guidelines addressing stereochemical topics concerning the submission of new drugs. Moreover, in 1993, the European Union Committee for Proprietary Medical Products (CPMP),[23] issued formal guidelines called "Investigation of Chiral Active Substance". Considering guidelines for Japan, they are essentially consistent with the recommended by the USA and EU.[24]

Since then, there was a large tendency to develop single enantiomeric drugs rather than racemates.[25] The number of worldwide new molecular entities (NMEs) approved from 2001 to 2010 is presented in **Figure 4** indicating that single enantiomers exceeded achirals while racemic drugs represented the minority category.[26]

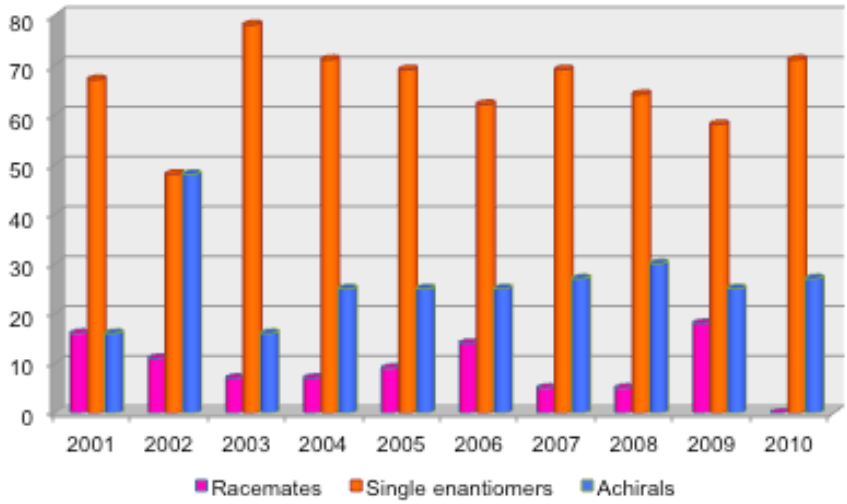


Figure 4: Number of worldwide-approved new molecular entities (NMEs) according to the chirality of the NME for the 2001–2010 period (adapted from [26]).

In addition to the regulatory requirements, also the advances in asymmetric syntheses and chiral separation methodologies, as well as the “chiral switch” phenomenum contributed to the increasing development of enantiomerically pure drugs rather than racemates.[10] Accordingly, for several years, the pharmaceutical industry has invested in the "chiral switch", developing single enantiomers from chiral drugs that have been developed, claimed, approved and marketed previously as racemates. These new enantiomeric drugs received additional patent protection and a new generic name.[2,27]

In recent years, most of the blockbuster drugs in the pharmaceutical market were single enantiomers. In fact, in 2015, according to IMS Health, three single enantiomeric drugs were on the top ten best-selling drugs.[28] The antiepileptic drug Lyrica® (pregabalin, Pfizer) was the sixth-most prescribed brand-name drug in that year, with about 10.1 million prescriptions. The antihyperglycemic drug Januvia® (sitagliptin, Merck) afforded 9.2 million prescriptions in USA, making it the eighth-most prescribed drug overall. The proton pump inhibitor Nexium® (esomeprazole, AstraZeneca), often indicated to treat acid reflux and heartburn, was the third-most prescribed drug in the USA, with 13.2 million prescriptions in that year.[28]

4. Enantiomeric purity

The need to generate both enantiomers in high enantiomeric purity for biological and pharmacological assays is crucial since the majority of the individual enantiomers exhibit distinct pharmacological profiles.[29]

The enantiomerically pure drugs can be obtained either by preparative resolution of a racemate or by enantioselective synthesis of the desired enantiomer.[30] While the enantioselective synthesis is useful when a large amount of one enantiomer is required, the enantioresolution more adequate to produce both enantiomers in the early stages of drug discovery process.[31,32] Enantioresolution by chromatography usually furnishes both enantiomers with high enantiomeric purity that is required for biological, pharmacological or toxicological evaluations.[25,33,34]

Regardless the strategy used to obtain the single enantiomers, it is crucial to evaluate their enantiomeric purity. The enantiomeric purity can be measured in a mixture of enantiomers. The composition is given by the enantiomeric excess (e.e.), which is an indicator of the enantiomeric purity.

There are several methods of measuring e.e. including nuclear magnetic ressonance (NMR) spectroscopy, optical rotation, liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), among others (**Figure 5**).

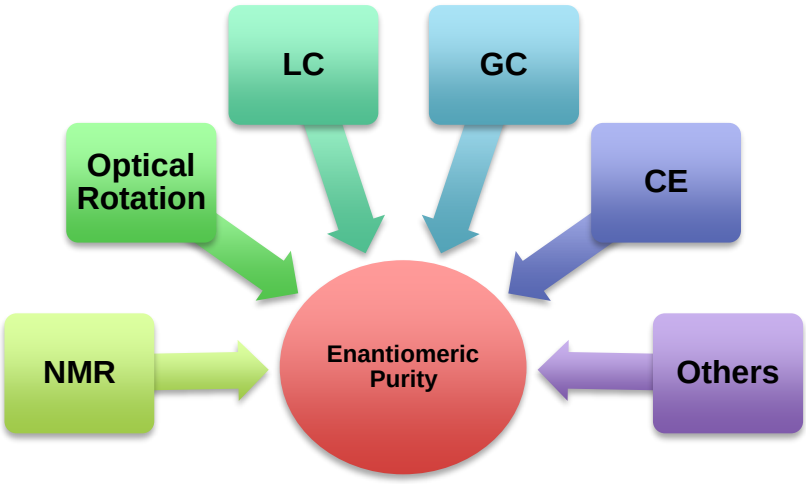


Figure 5: Common methods used to assess the enantiomeric purity.

Comparatively with the other methods, LC using chiral stationary phases (CSPs) is the most common method employed for the determination of e.e. (**Figure 6**)[25,35,36]

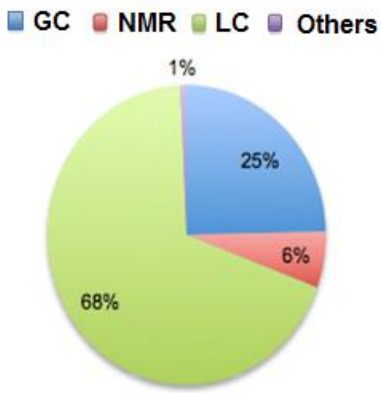


Figure 6: Distribution of the methods used for the determination of e.e., appeared in Journal of the American Chemical Society (2005) (adapted from [37]).

The e.e. can be measured by the percentage excess of the major enantiomer (E1) over the minor enantiomer (E2), determined by the relative percentages of the peak areas according to the following formula, where [E1] and [E2] are the peak area of each enantiomer.[38]

$$\%\ \text{Enantiomeric excess} = \frac{[\text{E1}] - [\text{E2}]}{[\text{E1}] + [\text{E2}]} \times 100$$

The closer the value is to 100%, the greater the enantiomeric purity.

Alternatively the e.e. can be expressed in terms of molar fraction of each enantiomer:

$$\%\ \text{Enantiomeric excess} = \frac{\text{moles of one enantiomer} - \text{moles of other enantiomer}}{\text{total moles of both enantiomers}} \times 100$$

Over the last few decades, a growing number of different types of CSPs became available namely Pirkle-type, ion- and ligand-exchange type, based on proteins, polysaccharides, macrocyclic antibiotics, synthetic polymers, cyclodextrins, crown ethers, cyclofructans, among others.[35] Nowadays, polysaccharide-based, macrocyclic antibiotics-based and Pirkle-type CSPs are pointed out as the most useful and broadly applied.[39,40]

5. Xanthone: a privileged structure

Chemically, xanthone (9H-xanthon-9-ones) is a symmetrical compound possessing a dibenzo-ɣ-pyrone framework (**Figure 7**). Most of derivatives of this oxygen-containing heterocyclic scaffold exhibit fluorescence which can be an advantage since they can be used as fluorescence probes.[30,41,42]

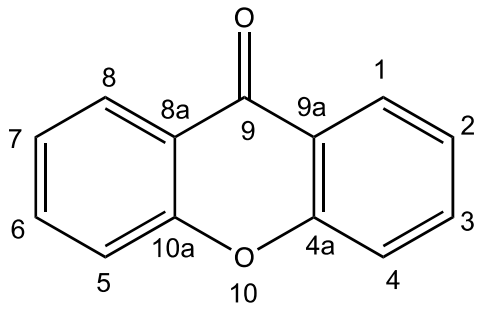


Figure 7: Xanthonic scaffold and numbering.

This family of compounds is very appealing in Medicinal Chemistry, being classified as privileged structures, because different patterns of substitution have been found leading to a considerable variety of compounds that are able to interact with several biological targets.[43-46] The xanthonic scaffold is not merely restricted to a pharmacophoric moiety role. In fact, it can also be a substituent group to modulate biological responses.[43]

Natural xanthones have been found in fungi, lichens and higher plants.[47,48] Most natural xanthones from higher plants are associated with the families *Clusiaceae* and *Gentianaceae.*[47] Natural xanthones can be classified in six groups, depending on the nature of the substituents in their scaffold: simple oxygenated xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, xanthone dimers and miscellaneous.[41,47,49,50]

The biosynthetic pathway of xanthones only allows the presence of certain groups in specific positions of the xanthonic scaffold, which is a limiting factor for the structural natural variation. For this reason, it was considered using total synthesis. Total synthesis allows the access to structures that otherwise could not be reached if it is used the natural product, as a launching platform for molecular modification. Moreover, a greater amount of compounds is obtained. The total synthesis can also help to rationalize the structure-activity relationship (SAR).[42,51,52]

Considering synthetic xanthones, they can have simple groups as substituents in the dibenzo-γ-pirone scaffold like hydroxyl, methoxyl, methyl, carboxyl, but can also have more complex substituents such as epoxide, azole, aminoalcohol, sulfamoyl, methylthiocarboxylic acid, and dihydropyridine.[43] A main objective of the synthesis of new derivatives is to develop more diverse and complex xanthones for biological activity even for SAR studies. The traditional methods to synthesize simple xanthones include: **(I)** *via* benzophenone intermediate - **(a)** the Grover, Shah, and Shah (GSS) reaction, and **(b)** condensation by Friedel-Crafts acylation, and **(II)** *via* diaryl ether intermediate (Ullman synthesis) (**Figure 8**).[51] Additionally, other several routes with higher yields and less drastic experimental conditions have been developed.[51,53]

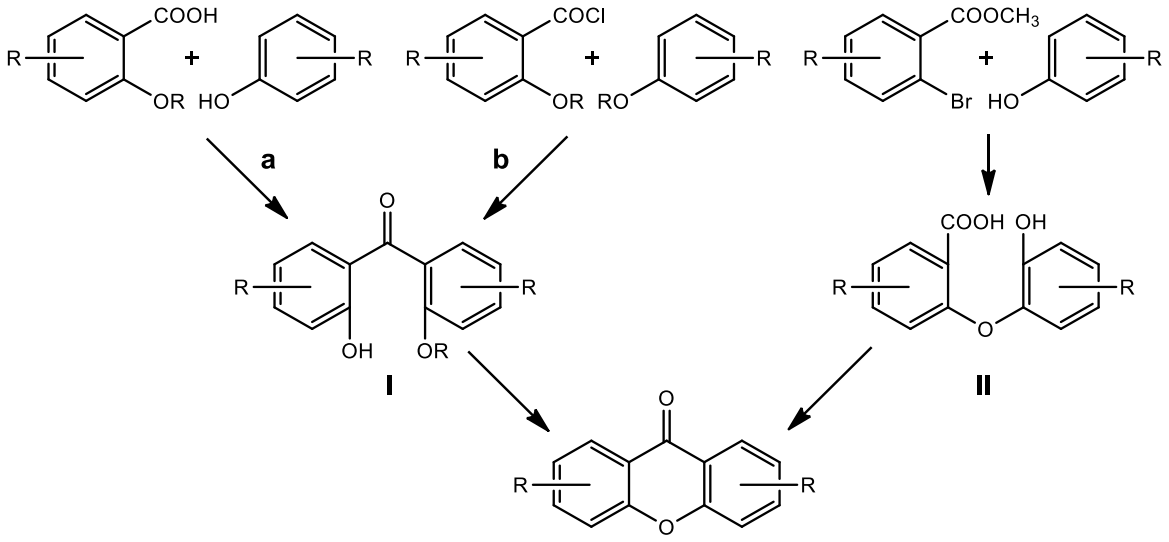


Figure 8: General methods for the synthesis of xanthone derivatives.

6. Chiral derivatives of xanthones

Recently, there has been an increase in the interest in new bioactive xanthones particularly in chiral derivatives of xanthones (CDXs). The importance of this class of compounds in Medicinal Chemistry, the promising biological/pharmacological activities of some chiral members of this family, the clinical advantages of a single enantiomer than a racemate, the scarce examples of synthetic CDXs described, are some of the reasons that can justify this trend.

6.1. Natural chiral xanthones

It is well known that many naturally occurring xanthones are chiral and present interesting biological activities. Some of them have been exhaustively studied, and have also been subsequently obtained by synthesis.[47] Historically, the first total synthesis of a naturally occurring xanthone was a euxanthone, described by Ullmann and Panchaud in 1906.[54]

Considering the large number of natural chiral xanthones, only some examples of compounds recently isolated will be presented highlighting their biological activities. The chemical structures of the natural CDXs and the associated biological activities are represented in **Appendix** (**Table 24**).

6.1.1. Sterigmatocystin and derivatives

Sterigmatocystin (**1**) was first isolated from the fungus *Aspergillus versicolor* in 1953,[55] and then from several other species over the years.[54] Its structure was only subsequently determined by a combination of spectral and chemical degradation techniques. Over the last fifty years, various derivatives of sterigmatocystin were isolated and characterized.[54] Among them, dihydrosterigmatocystin (**2**) and secosterigmatocystin (**3**) were isolated from the mangrove endophytic fungus ZSUH-36 in South China Sea.[56] In the same year, sterigmatocystin (**1**) and these two derivatives (**2**,**3**) were also found in fungus of *Kandelia candel.*[57]

The antitumor activity of sterigmatocystin (**1**) was first reported in 1975.[58] The results demonstrated that this compound has inhibitory effect of the growth of transplanted leukemias P-388 and L1210 in mice. In 2007, the biological activity of sterigmatocystin (**1**) and its derivatives dihydrosterigmatocystin (**2**) and secosterigmatocystin (**3**) against human DNA topoisomerase type (hTOP) was tested, and none of the three xanthones exhibited significant inhibitory effect.[57]

6.1.2. Asperxanthone

Asperxanthone (**4**) was isolated from the cultures of a marine-derived *Aspergillus sp*. collected in China sea water, in 2009.[59] This natural xanthone is structurally related to the difuranoxanthones (**1**) and (**2**), in the sterigmatocystin series. This compound was identified using 2D NMR techniques and FAB-MS (fast atom bombardment mass spectrometry) and was shown to have inhibitory activity against the tobacco mosaic virus.[54,59]

6.1.3. Chaetoxanthones

Chaetoxanthones A (**5**), B (**6**), and C (**7**) were isolated from the marine-derived fungus *Chaetomium sp.* in 2008.[60] Chaetoxanthones A (**5**) and B (**6**) are xanthones substituted with a dioxane/tetrahydropyran moiety rarely found in natural products. Chaetoxanthone C (**7**) is a chlorinated xanthone substituted with a tetrahydropyran ring. These chiral xanthones were tested in a series of *in vitro* bioassays for antiprotozoal activities and cytotoxic potency. Chaetoxanthone B (**6**) was found to be active against *Plasmodium falciparum* in the mid micromolar range, whereas Chaetoxanthone C (**7**) was active in low micromolar range against *Trypanosoma cruzi*, the causative organism of Chaga's disease.[54,60]

6.1.4. Psorospermin and derivatives

Psorospermin (**8**) was isolated from the bark and roots of the African plant *Psorospermum febrifugum* in 1980.[61,62] It is a natural fused tetracyclic xanthone containing two stereogenic centers with (2*R*,3'*R*)-stereochemistry and a reactive epoxide. The importance of the configuration and the functionality of the epoxydihydrofuran group for the *in vivo* activity have also been evaluated.[43,63] The total synthesis of psorospermin (**8**) was reported for the first time in 2005, by obtaining the xanthone skeleton by the method of Grover *et al.*[62] Psorospermin (**8**) was synthesized in thirteen steps with an overall yield of 1.7%. Psorospermin (**8**) revealed interesting biological activities showing antileukaemic, and also antitumor activity in several human cell lines.[43,62]

Additionally, the (*R,R*)-stereochemistry of psorospermin (**8**) gave optimum DNA alkylation and antitumor activity, although all four possible stereoisomers show topoisomerase II-dependent alkylation.[64] Molecular modeling rationalized this activity in terms of the energy of interaction with DNA and proximity of the epoxide ring CH_2 to N7 of guanine.[43,64]

Psorospermum febrifugum has yielded other compounds structurally similar to

psorospermin (**8**), without alkylating moieties, but with moderate activity against human tumor cell lines, such as psorofebrin (**9**) and isohydroxy-psorofebrin (**10**). Two ring-constrained analogues of psorospermin were also synthesized, namely, stereoisomer **11,** a ring-constrained (2*R*,3*R*)-form, and **12,** a ring-constrained (2*R*,3*S*)-compound.[64]

The chlorohydrin (**13**) retains the psorospermin-like DNA alkylation characteristics despite its rigid structure and high affinity for DNA. The chlorohydrin (**13**) and epoxide (**12**) showed increased cytotoxicity against a range number of human tumor cell lines, compared to isohydroxy-psorofebrin (**10**).[64]

Another study described the synthesis of two diastereisomeric pairs of *O*-5-methyl psorospermin and evaluation of *in vitro* activity against a range of solid and hematopoietic tumors. The diastereisomeric pair having the naturally occurring enantiomer (2*R*,3*R*) (**14**) was the most active across all the cell lines tested. In subsequent studies using all four isomers of *O*-5-methyl psorospermin, the order of biological potency was (2*R*,3*R*) > (2*R*,3*S*) = (2*S*,3*R*) > (2*S*,3*S*).[65] The compound (2*R*,3*R*) psorospermin (**14**) showed to be as effective as gemcitabine (chemotherapeutic drug) in slowing tumor growth *in vivo* in pancreatic cancer model.[65]

6.1.5. Xanthonolignoids

Xanthonolignoids are a natural class of compounds isolated from the plants of *Clusiaceae* family (*Guttiferae*).[66] They possess a phenylpropane skeleton linked to a xanthonic scaffold by a dioxane ring, formed by radical oxidative coupling.[67,68] Natural xanthonolignoids include kielcorins, cadensins, subalatin, calophyllumins and gemixanthone.[68] The first xanthonolignoid described was based in 2,3,4-trioxygenated xanthone isolated in 1969 from *Kielmeyera species*.[63]

Considering that xanthonolignoids are very interesting templates for molecular modifications, several xanthonolignoids were synthesized.[63,68,69] Initially the main goal for synthesis was to help in structure elucidation of this class of compounds but, then, also to improve biological and physicochemical properties.[68]

Both classic synthesis and biomimetic approaches have been used to obtain xanthonolignoids mainly kielcorin derivatives.[68] The total synthesis of kielcorin derivatives requires several steps and drastic reaction conditions while the biomimetic way is based on natural building blocks and is achieved by an oxidative coupling of a suitable dihydroxyxanthone and a cinnamyl alcohol derivative, in the presence of an oxidizing agent at room temperature.[68]

Pinto *et al.* have reported the first biomimetic synthesis of xanthonolignoids of the

kielcorin group. To obtain related bioactive compounds with a kielcorin framework, other constitutional isomers were synthesized by the same group (**15-19**)**.** Once again, the synthetic approach was based on a biomimetic pathway as a model, by oxidative coupling of coniferyl alcohol, with an appropriate xanthone.[69] In order to investigate the oxidative coupling reaction different oxidizing agents were used (e.g., Ag_2O, Ag_2CO_3, and $K_3[Fe(CN)_6]$).

Kielcorins (**15-19**) were evaluated for their *in vitro* effect on the growth of three human tumor cell lines, MCF-7 (breast), TK-10 (renal), UACC-62 (melanoma), and on the proliferation of human lymphocytes.[69] The growth inhibitory effect was moderate but dose-dependent, and influenced by the isomerism of the tested compounds. The compounds **15, 16** were the most active. The inhibition of human lymphocyte proliferation induced by phytohemagglutinin (PHA) was detected.[69] The high potency and selectivity observed for these compounds suggested that kielcorins may be an important model for developing potent and isoform-selective protein kinase C (PKC) inhibitors.[43] Accordingly, the kielcorins **15-19** revealed an effect compatible with PKC inhibition similar to that exhibited by the well-established PKC inhibitor chelerythrine.[70] Kielcorins **15** and **18** were evaluated and demonstrated protectives effects against *tert*-butylhydroperoxide-induced toxicity in freshly isolated rat hepatocytes.[71]

In order to study if the growth inhibitory effects of these kielcorins (**15-19**) were depended on the stereochemistry, analytical LC methods using four carbamate of polysaccharide derivative CSPs and multimodal elution conditions were developed for their enantioresolution.[72] The amylose *tris*-3,5-dimethylphenylcarbamate CSP was chosen to scale-up to the preparative resolution considering not only the highest enantioselectivity obtained for these chiral compounds but also due to low retention factors.[72] Consequently, the enantiomers of the kielcolins **15–18** were efficiently separated by chiral LC in multimilligram scale. A solid-phase injection system was developed and combined with a closed loop recycling system to increase the productivity and recovery of the preparative process.[31] The e.e. was also measured and was greater than 99% for each enantiomer, except for compound **15**.[31]

The inhibitory activity of the racemates **15-18** as well as of the corresponding enantiomers on *in vitro* growth of the human breast adenocarcinoma cell line MCF-7 was evaluated and compared. The most evident enantioselectivity was noticed between the racemate **16** (inactive) and the active enantiomers (+)-**16** and (−)-**16**.[31]

6.1.6. Tajixanthones

The tajixanthone family, curiously biosynthesized from a combination of xanthone and terpenoid-derived carbon units, includes the compound **20** and highly structurally related species shamixanthone (**21**) and their derivatives. Tajixanthone (**20**) and shamixanthone (**21**) were isolated for the first time in 1970, from cultures of *Aspergillus variecolor*, and then reisolated along the years.[54,73] Their structures were determinate by several techniques including NMR spectroscopy and chemical degradation.[73] The biosynthetic pathway to tajixanthone (**20**) and shamixanthone (**21**) was originally proposed based upon ^{13}C and ^{1}H feeding experiments.[54,74,75] Subsequently, shamixanthone (**21**) was synthesized by the epoxidation of tajixanthone (**20**) with triphenylphosphine selenide and trifluoroacetic acid.[74,75]

Tajixanthone (**20**) and shamixanthone (**21**) were tested against several tumor cell lines, including gastric, colon, lung and human hepatocarcinoma, in comparison with doxirubicin. Tajixanthone (**20**) showed widespread activity against all the tested cell lines.[76]

6.2. Synthetic chiral xanthones

Even though there is a large structural multiplicity of bioactive xanthonic compounds, there are only a few reports in literature about chiral synthetic derivatives. However, these CDXs seem to represent an attractive area of chemical research, since the described studies have shown that these xanthonic compounds exhibit important biological/pharmacological activities. Moreover, in some cases the biological activities are dependent on the stereochemistry of the CDXs.

For the last years, searching of new synthetic CDXs with potential biological activities has remained in the area of interest particularly of two research groups, Madalena Pinto's and Henry Marona's groups.

The chemical structures of the synthetic CDXs and the biological activities evaluated are included in **Appendix** (**Table 24**).

6.2.1. DMXAA analogues

DMXAA or dimethylxanthenone-4-acetic acid (**Figure 9**) is a simple carboxylated xanthone discovered in a SAR study involving a series of xanthone-4-acetic acids related to the parent compound flavone acetic acid.[77] DMXAA is a tumor vascular-disrupting agent leading to a fast vascular collapse and tumor necrosis by immunomodulation and cytokines induction, particularly of tumor necrosis factor-α (TNF-α). DMXAA also demonstrated a synergic effect when combined with chemotherapeutic agents.[78]

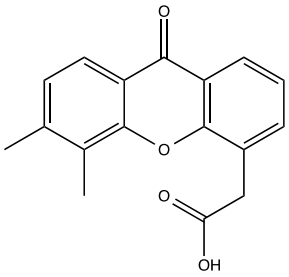


Figure 9: Chemical structure of DMXAA.

DMXAA is not a chiral xanthone, but its analogues **22** and **23** are. Both enantiomers of the DMXAA analogue 5-methyl-α-methyl-xanthone acetic acid, (**22a** and **22b**), were separated and tested *in vitro* and *in vivo* tumor assays. Both were active, but the *S*-(+)-enantiomer (**22b**) was much more dose-dependent than the *R*-(-)-enantiomer (**22a**). Rewcastle *et al*. suggested that the enantiomers have different intrinsic activities, rather than differing in their metabolism.[43,79]

6.2.2. Alkanolic chiral derivatives of xanthones

Pinto *et col.* have a vast experience in synthesis and biological evaluation of xanthone derivatives[30,80-82], and recently reported the synthesis of a library of CDXs in an enantiomerically pure form (**24-38**).[30,34,72] Among the synthesized CDXs, the compounds **25-30** and **33-38** are alkanolic, with the exception of CDXs **24**, **31** and **32**.

The synthesis of all the CDXs (**24-38**) were achieved through the coupling of two carboxyxanthone derivatives, with both enantiomers of eight commercially available chiral building blocks, using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent. TBTU has been widely used as efficient reagent for the synthesis of different classes of compounds such as peptides,[83] amides, phenylhydrazides,[84] esters. However, it was used for the first time to synthesize CDXs. The coupling reactions with the chiral blocks were performed at room temperature, showing short reactions times and excellent yields (ranging from 94% to 99%).[30,82] Carboxyxanthone derivatives demonstrated to be suitable molecular basis to perform molecular modifications to obtain chiral derivatives. The commercial chiral blocks used, such as amines, amino alcohols, and amino esters, included both enantiomers of enantiomerically pure building blocks with no tendency towards racemization or enantiomeric interconversion, and having a primary amine as reactive group for amide formation.[30] The synthetic methodology used to obtain the referred CDXs provided to be very efficient, broad-scope applicability, inexpensive and operationally simplest. Moreover,

the synthesis of CDXs may be easily scaled-up for both enantiomers in order to obtain available quantities for biological and pharmacological assays.[30]

LC using different types of CSPs namely polysaccharide-based,[34] macrocyclic antibiotics,[85] and Pirkle-type[82] was used to determine the enantiomeric purity of the synthesized CDXs. The enantioselective LC method using polysaccharide-based CSPs under multimodal elution conditions afforded very high resolutions with short chromatographic runs. The best performances were achieved on amylose *tris*-3,5-dimethylphenylcarbamate stationary phase under polar organic elution conditions. The resolution achieved allowed the determination of the e.e. for all CDXs, affording values higher than 99%. This study also explored the influence of different mobile phases as well as of the structural features of the CDXs on their chiral discrimination by the polysaccharide-based CSPs.[34]

Considering the macrocyclic antibiotic CSPs, the best enantioselectivity and resolution were achieved on Chirobiotic R and Chirobiotic T CSPs under normal elution conditions. The optimized chiral LC conditions were successfully employed for the accurate determination of the enantiomeric purity of eight enantiomeric mixtures of CDXs (**25-28**), always higher than 99%. Under these elution conditions the (*R*)-enantiomer of all the CDXs evaluated was the first to elute.[85]

Regarding the Pirkle-type CSPs, the (*S*,*S*)-Whelk-O1 CSP showed the best performance for the resolution of the CDXs evaluated, presenting very high enantioselectivity for CDXs with aromatic group linked to the chiral moiety.[86] Polar organic elution mode presented the best chromatographic parameters allowing good resolutions and lower run time.

Both enantiomers of the synthesized CDXs (**24-38**) were evaluated for their effect on the *in vitro* growth of three human tumor cell lines, namely A375-C5 (melanoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer).[30] The results obtained demonstrate that some CDXs exhibited interesting growth inhibitory effects on the tumor cell lines. The most active CDX in all human tumor cell lines was compound **30c**.[30] Furthermore, it is important to highlight that the effects on the growth of the human tumor cell lines were attributed not only to the nature and positions of substituents on the xanthonic scaffold, but also, in some cases, were associated with the stereochemistry of the CDXs concerning enantioselectivity results. Interesting examples of enantioselectivity were observed between the enantiomeric pairs of CDXs **24a** and **24b**, **26a** and **26b**, **30c** and **30d**.[30] These promising results were an inspiration for our group to enlarge the library of bioactive CDXs.

Taking into account that these CDXs have molecular moieties structurally very

similar to local anesthetics, the ability to block compound action potentials (CAP) at the isolated rat sciatic nerve was also investigated. In fact, these compounds are structurally similar to dibucaine, lidocaine, bupivacaine with which they might share common molecular moieties, and, consequently, the same pharmacophore. The absence of a tertiary amine is the most significant difference regarding the pharmacophoric descriptors for local anesthetics. The CDXs **24b, 25b** and **26b** were chosen for biological evaluation and the results suggested that the nerve conduction blockade might result predominantly from an action on Na^+ ionic currents. It was also investigated if the CDXs could prevent hypotonic haemolysis on rat erythrocytes. However, data suggested that CDXs **24b**, **25b** and **26b** caused no significant protection against hypotonic when applied in concentrations high enough to block the sciatic nerve conduction in the rat.[82]

Considering the inspiring evolution of CSPs based on small molecules developed by Pirkle and co-workers,[20] Pinto *et col.* selected three CDXs (**25b**, **27a**, **31b**) and developed and patented new CSPs.[87] The CSPs obtained are based on a completely different type of small molecules from those commercial available. Accordingly, the CDXs when being connected to a chromatographic support may consist of good CSPs for LC due to the structural features of the xanthonic scaffold which gives the possibility to perform different types of interactions (hydrogen, dipole–dipole and π–π), and the intrinsic enantioselectivity of the chiral moiety substituent.[20] The enantioselective capability of these CSPs was evaluated by LC under multimodal elution conditions using several chemical classes of chiral compounds, showing promising results.

As referred before, Marona *et col.* also described the synthesis and biological activity evaluation of a series of alkanolic CDXs (**39-75**, **102-107**).[44,88-93] CDXs **39-54**, **64-69** were synthesized by condensation of an appropriate 2-bromomethylxanthone or 2-bromomethyl-7-chloroxanthone with the corresponding aminoalkanol in toluene, in the presence of anhydrous potassium carbonate.[44,88,94] Some of them were isolated, characterized and examined as salts. The exchange of secondary amino group of compounds **48, 48a** and **48b** for a tertiary amino group (**53, 53a** and **53b**) was generated by reductive *N*-methylation.[44]

Compounds **55-63** were synthesized by the amination of (+-)-3-(2,3-epoxy)-propoxy)-xanthone in *n*-propanol, or 2-methyl-6-hydroxyxanthone using propylene epichlorohydrin, in the presence of sodium hydroxide and water.[91]

The synthesis of compounds **70-75** evolved as a multi-step process. At first, substituted benzoic acid reacted with 2- or 4-methylphenol in two steps involving Ullmann condensation and electrophylic addition. The intermediate methyl derivatives of substituted xanthone were used in the reaction with *N*-bromosuccinimide giving

appropriate bromide derivatives. The last step comprised an aminolysis by means of appropriate aminoalkanol carried out in toluene in the presence of anhydrous K_2CO_3.[95]

All the synthesized CDXs (**39-75**, **102-107**) were evaluated for anticonvulsant activity.[44,88-91,93,94,96-98] The studies involved three kind of tests: maximal electroshock-induced seizerus (MES), subcutaneous pentetrazole seizure threshold (scMet), and neurological toxicity (TOX).

In one of the first MES assays in mice, 2-amino-1-propanol-, 1-amino-2-propanol and 1-amino-2-butanol derivatives of 6-methoxy- or 6-chloroxanthone were the most interesting compounds. In fact, the results indicated that compound **43** was the most active.[44] Further study compared the anticonvulsant activity of CDX **43** (racemate) with the single enantiomers (**43a, 43b**). All the compounds showed excellent results, and no significant differences were observed in the anticonvulsant activity of the single enantiomers compared with the racemate.[99]

Additionally, the enantiomeric purity of **43a** and **43b** was determined by liquid chromatography–mass spectrometry method with an electrospray ionization interface (ESI-LC/MS). The separation of the two enantiomers (**43a** and **43b**) was carried out on the commercially available cellulose *tris*-(3,5-dimethylphenyl carbamate) CSP, Chiralcel® OD-RH, giving e.e. values higher than 99.9%.[90]

Interesting anticonvulsant results were also observed with alkanolic chiral derivatives of 7-chloroxanthone (**48** and **53**) which displayed anti-MES activity corresponding with that for phenytoin, carbamazepine and valproate.[88] Moreover, it is important to highlight that in this study some cases of enantioselectivity were observed. For example, although enantiomers **48a** and **48b** showed anticonvulsant activity, the (*S*)-enantiomer (**48b**) was more neurotoxic. Furthermore, considering the compound **53** (racemate), the (*R*)-enantiomer (**53a**) in comparison to (*S*)-enantiomer (**53b**) showed higher anticonvulsant activity.[88,96] A further study including chiral aminoalkanol derivatives substituted in position 2 of xanthonic scaffold (structures not shown) also emphasized the importance to examine biologically enantiomers rather racemates.[97]

Additionally, a structure-anticonvulsant activity relationship study was described including series of aminoalkanol derivatives of 6-methoxy- or 7-chloro-2-methylxanthone as well as 6-methoxy-4-methylxanthone (**71-75**).[95] All the compounds showed activity in the MES screen which is recognized as one of the two most widely used seizures models for early identification of candidate anticonvulsants. The tested compounds were evaluated in the form of racemic mixture and some additionally in the form of single enantiomers to determine stereochemistry and activity relationship. In fact, as demonstrated before,[95] stereochemistry is one of the factors that can potentially

influenced anticonvulsant activity of the CDXs. However, considering anti-MES activity it was not possible to establish relationship between stereochemistry and anticonvulsant properties because all sets of compound gave different results. Racemate and enantiomers showed either similar results or diverged in duration of activity or lower effective doses. However, the anticonvulsant activity was associated with both aminoalkanol type and respective configuration as well as the place of substitution in the xanthonic scaffold.[95]

The overall results from several studies of Marona *et col.* [44,88-91,94,96-98] are quite encouraging and suggested that in the group of xanthone derivatives new potential anticonvulsants might be found.

Some of alkanolic CDXs were also evaluated for cardiovascular activity,[91,94,98,100] including antiarrhythmic, hypotensive, $_1$- and β_1-adrenergic blocking activities, effect on the normal electrocardiogram and influence on central nervous system (CNS). Among the investigated compounds, some of them exhibited significant antiarrhythmic and/or hypotensive activity. For example, compounds **105** and **106** revealed the strongest anti-arrhythmic activity in the adrenaline-induced model of arrhythmia. Additionally, compound **106** was also the most potent concerning hypotensive activity.[93]

The effects on platelet aggregation of some racemic CDXs **39-41**, **55** and **56**, and the enantiomeric pure CDX **53a** were evaluated and showed interesting results. The most active and promising compound was **53a** which nearly completely inhibited the thrombin aggregation concentration (TAC).[101] The results indicate that the presence of the 2-*N*-methylamino-1-butanol at position 2 and the chloride atom at the 7-position of the xanthonic scaffold promotes antiplatelets activity.

Alkanolic CDXs **39**, **42**, **43, and 64** were used to assess mutagenic and antimutagenic activity in assays using the *Vibrio Harvey.*[102] According to the results obtained, the most beneficial mutagenic and antimutagenic profiles were observed for compound **64**. A modification of the chemical structure of compound **64** by the replacement of the hydroxyl group by a chloride improved considerably the antimutagenic activity. Thus, antimutagenic potency reached a maximum with the presence of tertiary amine and one chloride atom in the side chain. Minimal activity was showed to compound **43** and no antimutagenic activity was observed for compound **39**.[102]

6.2.3. Chiral xanthones containing piperazine moieties and analogues

Several CDXs containing piperazine moieties (**78-81**, **83-101**) and analogues (**76**, **77**, **82**) were synthesized and their biological activity evaluated by Marona's group.[45,46,89,91,92,101,103-105]

The 2-hydroxyxanthone was the building block to synthesize compounds **78-85** using epichorhydrin in presence of pyridine.[89] Compounds **86-92** were synthesized by amination of 2-(2-bromoethoxy)-9*H*-xanthen-9-one and derivatives in *n*-propanol or toluene in the presence of K_2CO_3.[103] The compounds **93-95** were obtained by amination of 4-[(2,3-epoxy)propoxy]xanthone with appropriate 1-piperazine derivatives in *n*-propanol.[45] Chiral compounds **96-101** were obtained by amination of respective parent compounds [105] with appropriate amines in *n*-propanol. In addition, compound 98 was obtained from compound **96** by acetylation. In order to optimize synthetic methodologies, CDX **96** was obtained using an alternative method including (*R,S*)-4-(3-chloro-2-hydroxypropoxy)-9H-xanthen-9-one as intermediate.[105]

CDXs **78-85** were evaluated for anticonvulsant activity in the MES- and subcutaneous pentylenetrazole-induzed seizures in mice and rats.[89] Among them, the most promising compound was CDX **79** which was active in both the anticonvulsant tests.

Moreover, the influence on the platelet aggregation of CDXs **76**, **77**, **80** and **82** was evaluated by using adenosine-5'-diphosphate (ADP), sodium arachidonate (AA) or thrombin as the aggregating agents.[101] CDXs **77** and **82** were active, inducing 80-90% inhibition of thrombin-stimulated platelet aggregation.

Considering that the xanthone itself proved to possess vasorelaxating properties in thoracic aorta isolated from rats [106] and the strongest hypotensive effects were observed for compounds containing piperazine moiety,[105] several compounds that combining xanthone nucleus and piperazine rings (**86-101**) were evaluated for anti-arrhythmic and/or antihypertensive activities.[45,46,103,105] It is important to emphasize that CDXs **86** and **89** demonstrated to possess significant anti-arrhythmic activity in the adrenaline-induced model of arrhythmia.[103] The strongest hypotensive activity which persisted for 60 minutes was also associated to compound **86**.

Additionally, in another study related to the some biological activities, compounds **93** was the most promising in its effect on circulatory system. Moreover, this CDX diminished arterial blood pressure by about 40% during one hour.[45]

A recent cardiovascular activity study of several CDXs (**96-101**) was described including the following pharmacological experiments: the binding affinity for adrenoceptors, the influence on the normal electrocardiogram, the effect on the arterial blood pressure and prophylactic antiarrhythmic activity in adrenaline induced model of arrhythmia (rats, iv).[105] The CDXs **96** and **97** revealed to act as potential antiarrhythmics in adrenaline induced model of arrhythmia in rats after intravenous injection. The results obtained were quite promising and suggested that in the group of xanthone derivatives new potential antiarrhythmics and hypotensives might be found.

A series of some chiral derivatives of 2-xanthone with piperazine moiety (**78-85**) was evaluated for their activity against *M. tuberculosis*. The highest level of activity against *M. tuberculosis* was observed for compound **81**, which exhibited 94% growth inhibition. This compound was also examined for its anti- *M. avium* activity as well as cytotoxicity, showing insignificant anti *M. avium* activity and cytotoxic effects.[104]

In summary, CDXs, from both natural and synthetic origin, represent an attractive area of in Pharmaceutical Medicinal Chemistry concerning their broad spectrum of biological/pharmacological activities. Natural products may undoubtedly correspond to an important novel source of bioactive chiral xanthones of great interest, with synthetic methodologies also furnishing interesting CDXs, particularly single enantiomers. Moreover, naturally occurring chiral xanthones have been subsequently obtained by synthesis. CDXs revealed interesting biological activities, such as antitumor, antiarrhythmic, anticonvulsant, antibacterial, antiplatelet, and antihypertensive, among others. The results are encouraging and suggested that in the group of xanthone derivatives new potential antitumor, anticonvulsant and antiarrhythmic agents might be found. Moreover, interesting examples showed that biological activities were dependent on the stereochemistry of the CDXs. The referred cases highlight the essential role of development of efficient methodologies to synthesize bioactive chiral xanthonic derivatives in high yields and with high enantiomeric purity.

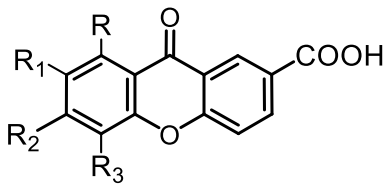
Chapter 2:

Results and Discussion

1. CHEMISTRY

1.1. Synthesis of carboxyxanthone derivatives

Carboxyxanthone derivatives (XCars) are not only interesting bioactive compounds [79,107] but they are also very important chemical substrates to obtain new chiral derivatives.[90] Herein, the total synthesis of three different XCars (**Figure 10** and Erro! A origem da referência não foi encontrada. of **Appendix**), namely 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2**, 108**), 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-3**, 109**) and 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5**, 110)**, was carried out by a multi-step pathway. To our knowledge, XCar-5 **(110)** is described here for the first time. All the XCars were obtained via modified Ullmann reaction with the formation of diaryl ether intermediates starting from an aryl bromide and a phenol.

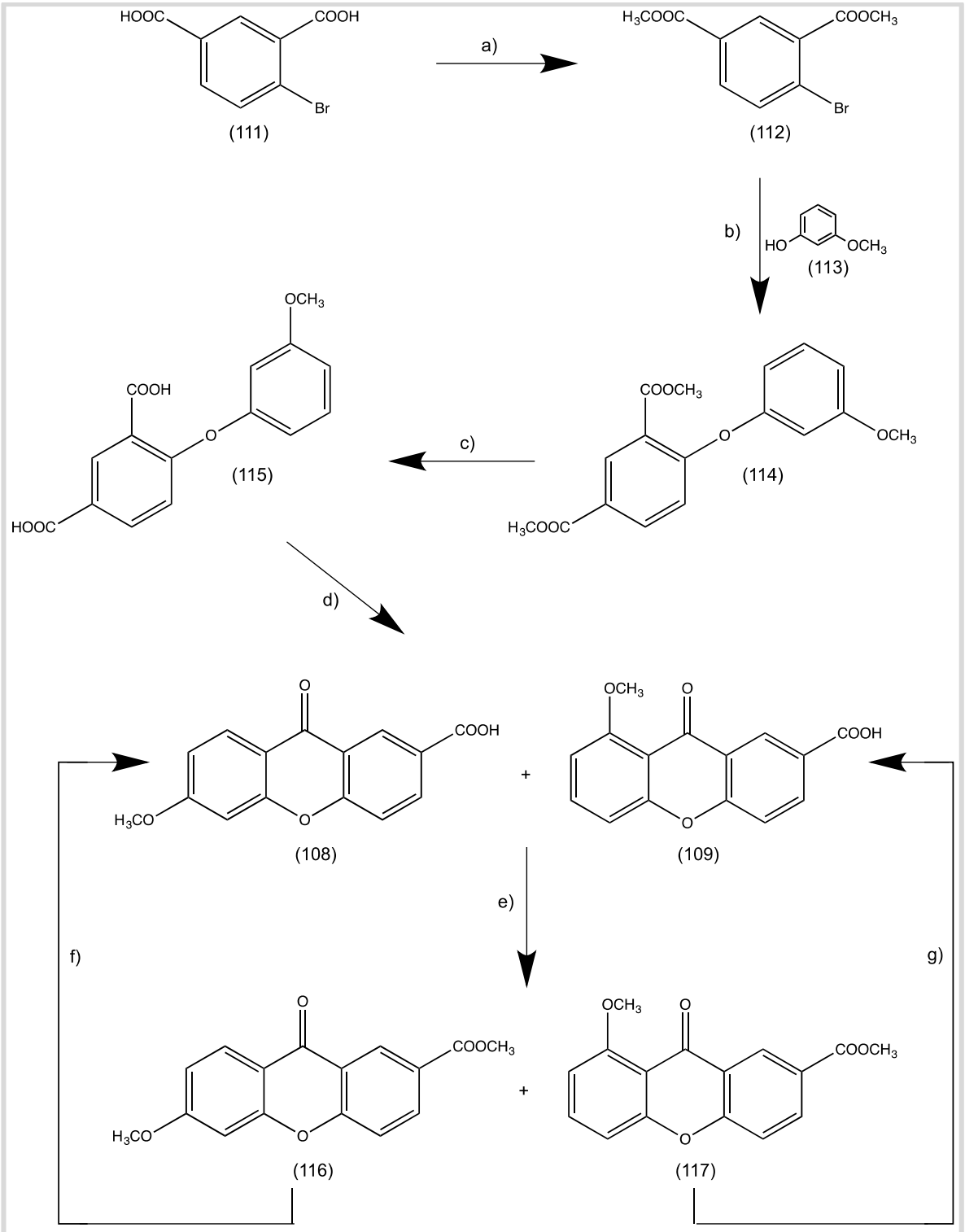


	R	R_1	R_2	R_3
XCar2	H	H	OCH_3	H
XCar3	OCH_3	H	H	H
XCar5	H	CH_3	H	CH_3

Figure 10: Chemical structures of XCar-2 **(108)**, XCar-3 **(109)** and XCar-5 **(110)**.

1.1.1. Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, 108) and XCar-3 (109)

The multi-step pathway for synthesis of XCar-2 **(108)** and XCar-3 **(109)** is illustrated on **Scheme 1**.



Reagents and conditions: a) Methanol, H_2SO_4, reflux, 17 h; **b)** CuI, K_3PO_4, Picolinic acid, Dimethyl sulfoxide, N_2, 80 ºC, 28 h; **c)** Methanol/Tetrahydrofuran (1:1 v/v), 5M NaOH, room temp.,18 h; **d)** P_2O_5, CH_3SO_3H, room temp., 22 h; **e)** Methanol, H_2SO_4, reflux, 19 h; **f)** and **g)** Methanol/Dichloromethane (1:1 v/v), 5M NaOH, room temp., 22 h.

Scheme 1: Total synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar2, 108)** and 8-methoxy-9-oxo- 9*H*-xanthene-2-carboxylic acid **(XCar3, 109)**.

The two carboxyxanthones, XCar-2 (**108**) and XCar-3 (**109**), were synthesized *via* Ullmann reaction, with the formation of the diaryl ether intermediate **114** from dimethyl 4-bromoisophthalate (**112**) and 3-methoxyphenol (**113**). The aryl bromide **112** was previously prepared from the corresponding carboxylic acid (**111**) by Fisher esterification (reaction **a**).

The Ullmann condensation (reaction **b**) between **112** and **113** under the catalytic action of CuI and picolinic acid in combination with K_3PO_4 in dimethyl sulfoxide (DMSO) provided the diaryl ether **114**. This coupling reaction was carried out at 80 °C under nitrogen atmosphere for 28 h to give the compound **114** in excellent yield (96%). The K_3PO_4/DMSO was used as a base/solvent combination and picolinic acid as a ligand for copper.

In a previous work, our group used *N*,*N*-dimethyl glycine and the stronger base Cs_2CO_3 to promote the Ullmann coupling reaction between the same building blocks using higher reaction temperature, in shorter reaction time but with lower yield.[82] As expected, this reaction had also better yield than the traditional method using copper-catalyzed in polar solvents typically pyridine and K_2CO_3 at high reaction temperature as described before.[107]

The reaction conditions, time and yield of the three Ullmann reactions are shown in **Table 2**. Consequently, the optimization performed on Ullmann reaction using a different solvent, catalysts and reaction conditions was effective due to the lower reaction temperature and remarkable yield. Additionally, picolinic acid is economically more attractive.

The diaryl ether (**114**) was dissolved in a mixture of tetrahydrofuran/methanol and 5M NaOH solution in order to hydrolyse the methyl esters (reaction **c**), affording the compound **115**. An intramolecular acylation of diaryl ether **115** (reaction **d**), using phosphorus pentoxide and methane sulfonic acid (Eaton's reagent) at room temperature afforded two compounds, the XCar-2 (**108**) and its isomer XCar-3 (**109**) in different yields. The separation of the two isomers was achieved through a Fisher esterification (reaction **e**) followed by flash column chromatography. After separation, the methyl esters of compounds **116** and **117** were hydrolysed (reactions **f** and **g**, respectively) in alkaline medium yielding the carboxyxanthones XCar-2 (**108**) and XCar-3 (**109**), respectively. This synthetic pathway has already been described,[82] but here was modified regarding reaction conditions and purification techniques.

Table 2: Optimization of Ulmann reaction.

Reagents	Time and temp.	Yield
CuI, Pyridine, K_2CO_3	24 h, 115 ^{0}C	32% [107]
N,*N*-Dimethyl glycine, CuI, Cs_2CO_3, Dioxane, N_2	14 h, 90 ^{0}C	54% [82]
CuI, K_3PO_4, Picolinic Acid, DMSO, N_2	24 h, 80 ^{0}C	96%

1.1.2. Synthesis of 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, 110)

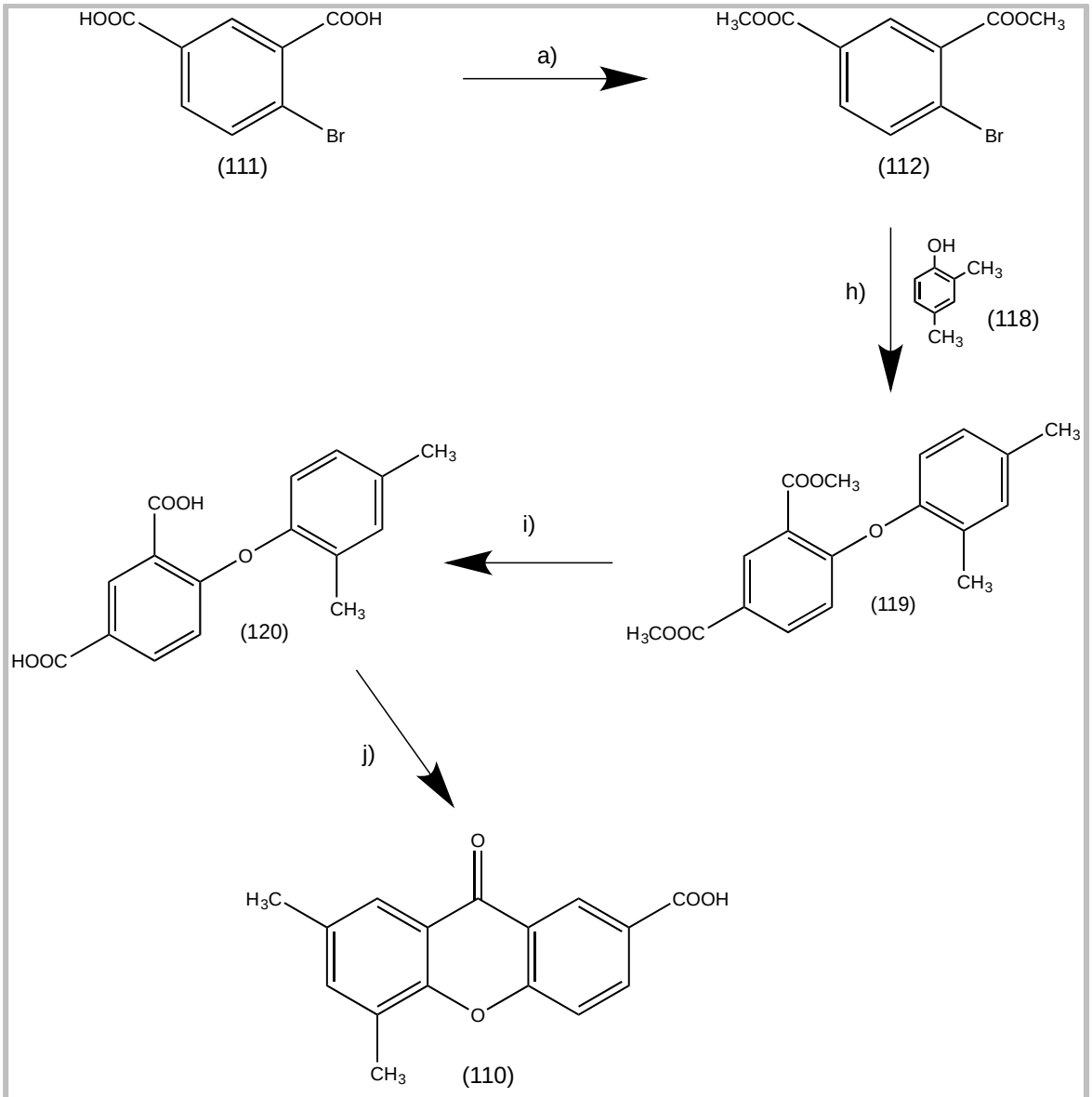
The multi-step pathway for synthesis of XCar-5 **(110)** is illustrated on **Scheme 2.**

The first step to synthesize XCar-5 (**110**) comprised a Fisher esterification reaction (reaction **a**), common to the synthetic pathway of XCar-2 (**108**) and XCar-3 (**109**) (**Scheme 1**).

The Ullmann condensation between the synthesized aryl bromide (**112**) and 2,4-dimethylphenol (**118**) (reaction **h**), under the catalytic action of CuI and picolinic acid in combination with K_3PO_4 in DMSO, provided the diaryl ether **119** (**Scheme 2**). Once again, the coupling reaction was carried out at 80 °C under nitrogen atmosphere for 28 h to give the compound **119**.

The diaryl ether **119** was dissolved in a mixture of tetrahydrofuran/methanol and 5M NaOH solution to hydrolyze the methyl esters (reaction **i**), affording the compound **120**.

An intramolecular acylation of the diaryl ether **120**, using phosphorus pentoxide and methane sulfonic acid at room temperature (reaction **j**) afforded the XCar 5 (**110**).



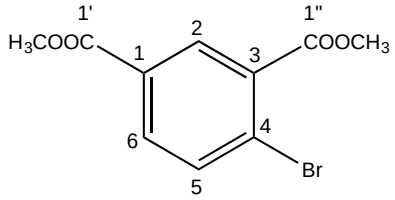
Reagents and conditions: **a)** Methanol, H_2SO_4, reflux, 17 h; **h)** CuI, K_3PO_4, Picolinic acid, Dimethyl sulfoxide, N_2, 80 ºC, 28 h; **i)** Methanol/Tetrahydrofuran (1:1 v/v), 5M NaOH, room temp., 18 h; **j)** P_2O_5, CH_3SO_3H, room temp., 48 h.

Scheme 2: Total synthesis of 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid **(XCar-5**, **110)**.

1.2. Structure elucidation of carboxyxanthone derivatives and their intermediates

All the XCars and their intermediates were characterized by IR, ^{1}H and ^{13}C NMR. (**108**,**109**,**112**,**114-117** also characterized by MS). XCar 5 (**110**) was also characterized by HMBC and HSQC techniques.

1.2.1. Structure elucidation of dimethyl 4-bromoisophthalate (112)



(**112**)

Dimethyl 4-bromoisopthalate (**112**) resulted from the esterification of 4-bromoisophthalic acid (**111**). The IR, ^{1}H and ^{13}C NMR, and MS allowed the structure elucidation of compound **112**.

Comparing the IR spectra of compounds **112** and **111** (**Table 3**), it is important to highlight that two broad bands at 2625 and 2880 cm^{-1}, corresponding to O-H bond of carboxylic acids, are only observed on spectrum of compound **111**. Moreover, the shift of the band at 1687 cm^{-1} (C=O bond of carboxylic acid) to 1754 cm^{-1} (C=O bond of ester) confirmed that ocurred transformation from COOH to $COOCH_3$.

Table 3: IR data of 4-bromoisophthalic acid (**111**) and dimethyl 4-bromoisophthalate (**112**).

Bond	υ (cm^{-1})	
	(**111**)	(**112**)
C=O	1687	1754
C-O	1255	1309 and 1253
O-H	2625 and 2880	--
C=C (aromatic)	930	929

Considering the ^{1}H NMR spectrum of compound **112** (**Figure 11**) it is important to highlight the presence of two singlets, at 3.95 ppm and 3.93 ppm, with integration for three protons each, corresponding to the protons of two $COOC\underline{H}_3$. In the ^{13}C NMR spectrum of compound **112** it is possible to observe the presence of two signals at 52.7 and 52.5 ppm corresponding to the carbons $C(1'')OO\underline{C}H_3$ and $C(1')OO\underline{C}H_3$, respectively.

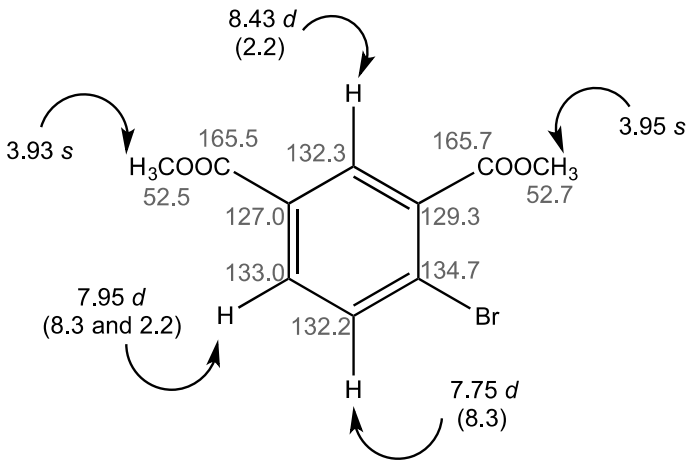
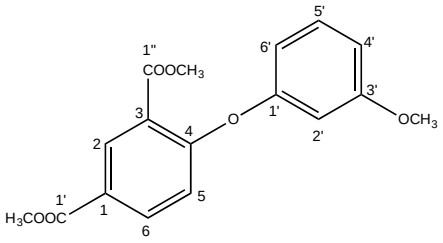


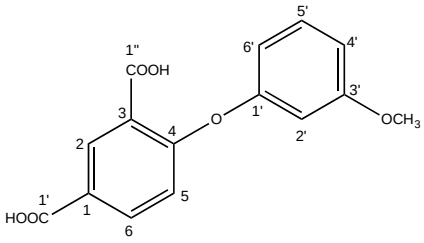
Figure 11: ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of dimethyl 4-bromoisopthalate (**112**).

MS spectra of compound **112** showed the following peaks and *m/z* values: 273 [M]+. (100), 256 (9), 240 (13), 221 (6), 209 (8), 203 (11). The presence of the respective molecular ion peak at the expected m/z values, confirmed the success of the reaction.

1.2.2. Structure elucidation of dimethyl 4-(3'-methoxyphenoxy)isophthalate (114) and 4-(3'-methoxyphenoxy)isophthalic acid (115)



(**114**)



(**115**)

Dimethyl 4-(3'-methoxyphenoxy)isophthalate (**114**) resulted from the Ullmann diaryl ether coupling of dimethyl 4-bromoisophthalate (**112**) and 3-methoxyphenol (**113**). The compound 4-(3'-methoxyphenoxy)isophthalic acid (**115**) resulted from the hydrolysis of the methyl esters of compound **114**. The IR, ^{1}H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **114** and **115**.

Comparing the IR spectrum of compound **114** (**Table 4**) with the spectrum of its precursor **112** (**Table 3**) showed the presence of the absortion bands corresponding to the C=O and to aromatic C=C in both spectra. The main difference important to highlight is

the presence of the absorption band C-O-C corresponding to the ether bond (1229 cm^{-1}) the IR spectra of both compounds (**112** and **114**).

Comparing the IR spectra of compounds **114** and **115** (**Table 4**), it can be seen the presence of the absorption bands corresponding to the C=C aromatic ring bond and to C-O-C ether bond in both spectra, with IR frequencies not significantly different. The presence of a broad absorption band at 2907 cm^{-1}, corresponding to the of O-H carboxyl acid bond, on spectrum of compound **115** confirmed that the reaction occurred successfully. Moreover, the shift of the band at 1719 cm^{-1} and 1724 cm^{-1} (C=O of $COOCH_3$) to 1680 cm^{-1} and 1694cm^{-1} (C=O of COOH) confirmed that occurred transformation from $COOCH_3$ to COOH.

Table 4: IR data of dimethyl 4-(3'-methoxyphenoxy)isophthalate (**114**) and 4-(3'-methoxyphenoxy)isophthalic acid (**115**).

Bond	υ (cm^{-1})	
	(**114**)	(**115**)
C=C (aromatic)	1613 and 1488	1601 and 1486
C=O	1724 and 1719	1694 and 1680
O-H	---	2907
C-O (ester)	1153	---
Ar-OCH_3	1274	1271
C-O-C	1229	1265

The ^{1}H and ^{13}C NMR data obtained for compounds **114** (**Figure 12**) and **115** (**Figure 13**) presented $\boldsymbol{\delta}$ values consistent to their proposed structures.

The ^{1}H NMR spectrum of compound **114**, compared to the spectrum of its precursor (**112**) (**Figure 11**), presented new signals such as one singlet at $\boldsymbol{\delta}$ 3.79 ppm, integration for three protons, corresponding to Ar-OCH_3, which confirmed the desired transformation.

Compounds **114** and **115** showed similar NMR profiles with respect to the aromatic protons with similar chemical shifts and coupling constants. The most significant difference in the ^{1}H NMR spectra is to the absence in compound **115** spectrum of two singlets at $\boldsymbol{\delta}$ 3.89 ppm and $\boldsymbol{\delta}$ 3.93 ppm, with the integration for three protons each, corresponding to the protons of the two $COOC\underline{H}_3$ methyl groups of compound **114**. This difference confirmed the success of the desired transformation.

The ^{13}C NMR spectrum of compound **114** compared to the spectrum of its precursor (**112**), presented a signal at δ 55.4 ppm, corresponding to the carbon of Ar-OCH_3 group, and associated to the signals of aromatic carbons, confirmed the desired transformation.

The most significant differences between the ^{13}C NMR spectra of compounds **114** and **115** correspond to the shift of carbons signals from δ 165.3 and 165.7 ppm (ester) to δ 179.3 ppm (carboxylic acid), and the absence, in the spectrum of compound **115**, of two signals at δ 52.2 and 52.3 ppm, corresponding to the carbons of the two $COOCH_3$ methyl groups.

The referred differences confirmed the success of the transformation.

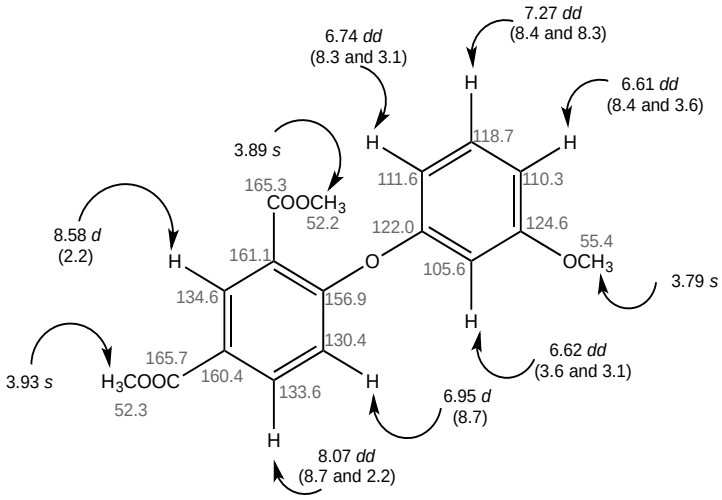


Figure 12: ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of dimethyl 4-(3'-methoxyphenoxy)isophthalate (**114**).

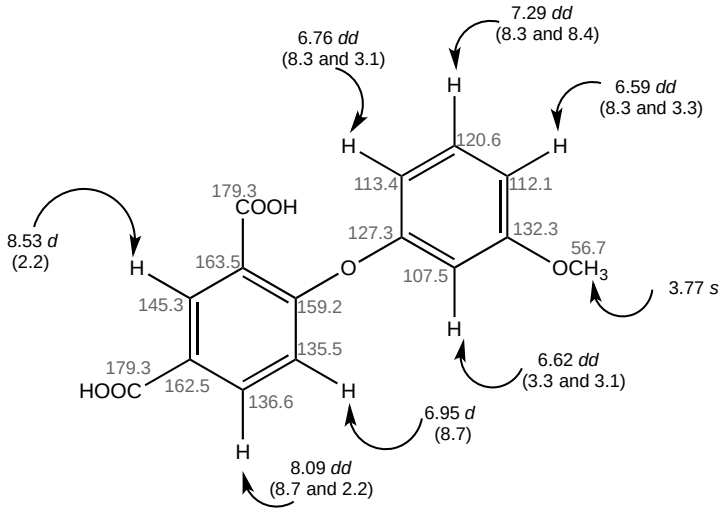
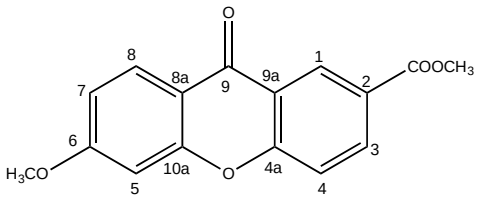


Figure 13: ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of 4-(3'-methoxyphenoxy)isophthalic acid (**115**).

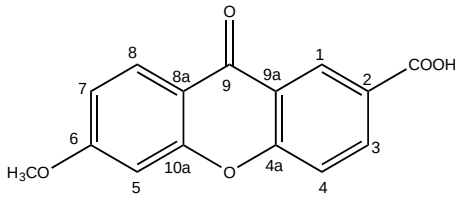
MS spectra of compound **114** showed the following peaks and *m/z* values: 316 [M]+. (100), 285 [M-OCH_3]+. (55), 253 (54), 242 (10), 225 (16), 213 (12), 198 (25), 138 (15), 127 (10), 92 (15), 83 (14), 64 (11). The intense signal at 316 corresponding to the peak of the molecular ion, the base peak is consistent with the proposed structure.

MS spectra of compound **115** showed the following peaks and *m/z* values: 288 [M]+. (100), 271 (10), 257 [M-OCH_3] +. (12), 227 (11), 199 (23), 165 (61), 124 (63), 92 (23), 77 (20), 64 (19). The molecular ion peak at 288, which also corresponded to the most intense peak of the spectrum is consistent with the proposed structure.

1.2.3. Structure elucidation of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (116) and 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar2, 108)



(**116**)



(**108**)

The 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, **108**) resulted from an intramolecular acylation reaction of the 4-(3'-methoxyphenoxy)isophthalic acid (**115**). Methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**116**) resulted from the esterification of XCar-2 (**108**). The IR, ^{1}H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **108** and **116**.

The IR spectra of compounds **108** and **116** (**Table 5**) showed the presence of the absorption bands corresponding to the of C=O and aromatic C=C bonds associated with the xanthonic sccafold. It is also important to point out the presence of the absorption band corresponding to of the Ar-OCH_3 bond at 1271 cm^{-1} for compound **108** and at 1270 cm^{-1} for compound **116**.

Tha main changes are: the shift of the band at 1610 cm^{-1} (C=O of COOH) to 1730 cm^{-1} (C=O of $COOCH_3$). As well as the absence of a broad band at 2901 cm^{-1} (OH of COOH) in IR spectrum of compound **116**. Considering that occurred transformation from COOH to $COOCH_3$.

Table 5: IR data of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, **108**) and methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**116**).

Bond	υ (cm^{-1})	
	(**108**)	(**116**)
O-H	2901	---
C=O (ester)	---	1730
C=O (ketone)	1687	1663
C=O (carboxylic acid)	1610	---
C=C (aromatic)	1575,1500, 1433	1581, 1467, 1438
Ar-OCH_3	1271	1270
C-O (ester)	---	1117
C-H (aromatic)	766	764

The ^{1}H and ^{13}C NMR spectra data of the xanthones **108** (**Figure 14**) and **116** (**Figure 15**) are consistent to the proposed structure for these compounds, and allowed not only to confirm the success of the transformation, but also to structurally elucidate them.

The analysis of ^{1}H NMR data of compounds **108** and **116** revealed that both compounds showed similar NMR profiles with respect to the six aromatic protons of the xanthonic scaffold and to the three protons of methoxyl group, with similar chemical shifts and coupling constants. Moreover, it is also important to note the presence in compound **116** spectrum of a singlet at δ 3.95 ppm, with the integration for three protons, corresponding to the COOC<u>H</u>$_3$ protons, which are absent in compound **108**.

The most significant differences between the ^{13}C NMR spectra of compounds **108** and **116** were associated to the signals of the carbons of the groups COOH and $COOCH_3$. The presence in the spectrum of compound **116** of the signal at δ 52.3 ppm corresponding to COO<u>C</u>H_3, confirmed the desired transformation from COOH to $COOCH_3$.

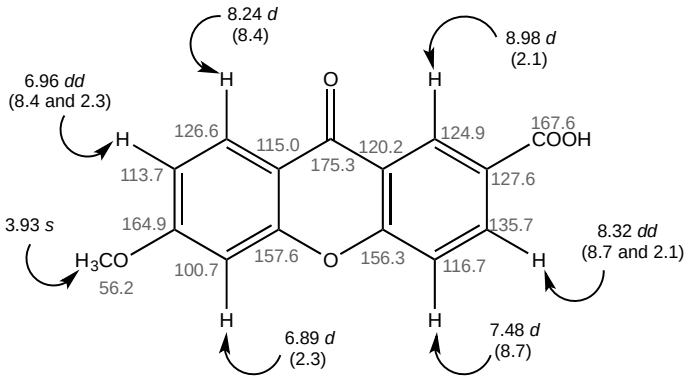


Figure 14: ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, **108**).

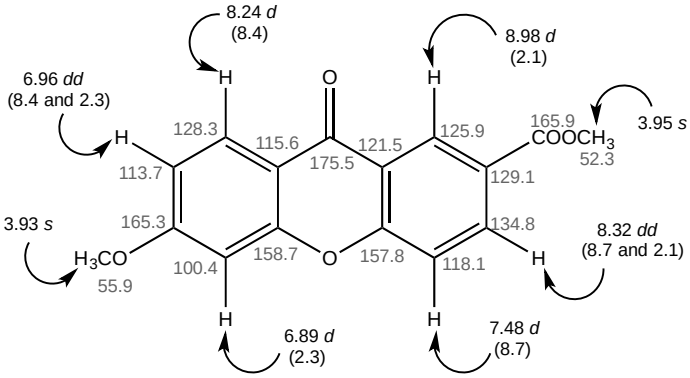
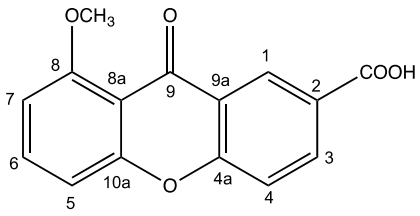


Figure 15: ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**116**).

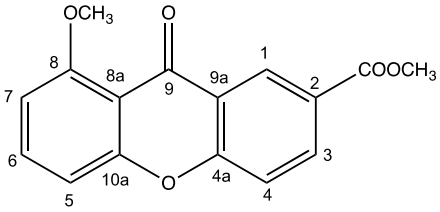
The MS spectra of the compound **108** showed the following peaks and *m/z* values: 270 [M]+. (100), 253 [M-OH]+. (26), 226 (27), 199 (15), 182 (15), 169 (8), 154 (7), 139 (7), 126 (17), 115 (10), 63 (16). The strong signal at 284, corresponding to the peak of the molecular ion obtained is consistent with the proposed structure.

The MS spectra of the compound **116** showed the following peaks and *m/z* values: 284 [M]+. (89), 253 [M-OCH_3] +. (100), 225 (32), 197 (14), 182 (30), 169 (17), 154 (16), 142 (14), 126 (28), 111 (12), 75 (12), 63 (14). The intense signal at 270, corresponding to the peak of the molecular ion obtained is consistent with the proposed structure.

1.2.4. Structure elucidation of 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar3, 109) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (117)



(**109**)



(**117**)

The 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-3, **109**) resulted from an intramolecular acylation reaction of the 4-(3'-methoxyphenoxy)isophthalic acid (**115**). Methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**117**) resulted from the esterification of XCar-3 (**109**). The IR, ^{1}H and ^{13}C NMR, and MS allowed the structure elucidation of compounds **109** and **117**.

The IR spectra of compounds **109** and **117** (**Table 6**) presented absorption bands characteristic of C=O and C=C bonds from the carbonyl group and aromatic rings, respectively, of the xanthonic scaffold. It is also important to point out the presence of the absorption band characteristic of the Ar-OCH_3 bond at 1266 cm^{-1} for compound **109** and at 1264 cm^{-1} for compound **117**.

Table 6: IR data of 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-3, **109**) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**117**).

Bond	**υ (cm^{-1})**	
	(**109**)	(**117**)
O-H	3460	---
C=O (ester)	---	1726
C=O (ketone)	1687	1668
C=O (carboxylic acid)	1663	---
C=C (aromatic)	1420, 1469, 1603	1431, 1480, 1611
Ar-OCH_3	1266	1264
C-O (ester)	---	1079
C-H (aromatic)	763	760

The comparison of IR spectra of both xanthones (**109** and **117**) revealed the shift of the band at 1668 cm^{-1} (C=O of $COOCH_3$) to 1687 cm^{-1} (C=O of COOH). It is also important to highlight the presence of large band at 3460 cm^{-1} corresponding to O-H (carboxylic acid) in xanthone **109**.

The ^{1}H and ^{13}C NMR spectra data are in agreement with the proposed structure for compounds **109** and **117** (**Figure 16** and **Figure 17**).

The analysis of ^{1}H NRM data of compounds **109** and **117** revealed that both compounds presented a singlet at δ 3.94-3.98 ppm, with integration for three protons, corresponding to the group Ar-O$C\underline{H}_3$ protons. Moreover, it is also important to note the presence in compound **117** spectrum of a singlet at δ 4.05 ppm, with the integration for three protons, corresponding to the group $COOC\underline{H}_3$ protons, which are absent in xanthone **109**.

The most significant differences between the ^{13}C NMR spectra of compounds **109** and **117** were related to the signals of the carbons of the groups COOH and $COOCH_3$. The presence in the spectrum of compound **117**, a signal at δ 52.4 ppm corresponding to $COO\underline{C}H_3$, and lower δ of signal to the carbonyl group of COOH compared to C=O from ester bond, confirmed the desired transformation.

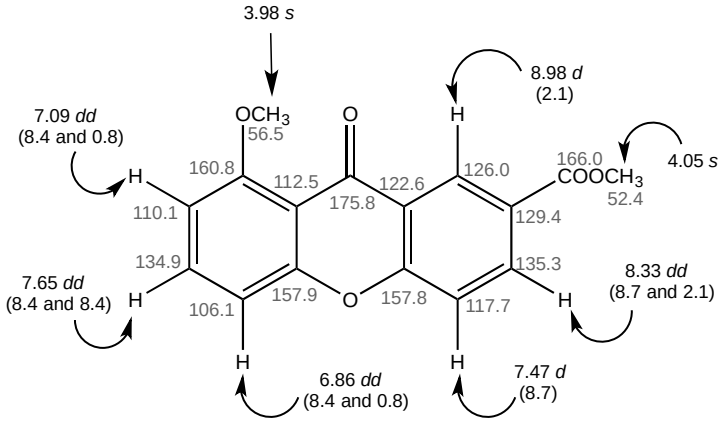


Figure 16: ^{1}H NMR ($CDCl_3$, 300.13 MHz) and ^{13}C NMR ($CDCl_3$, 75.47 MHz) of methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**117**).

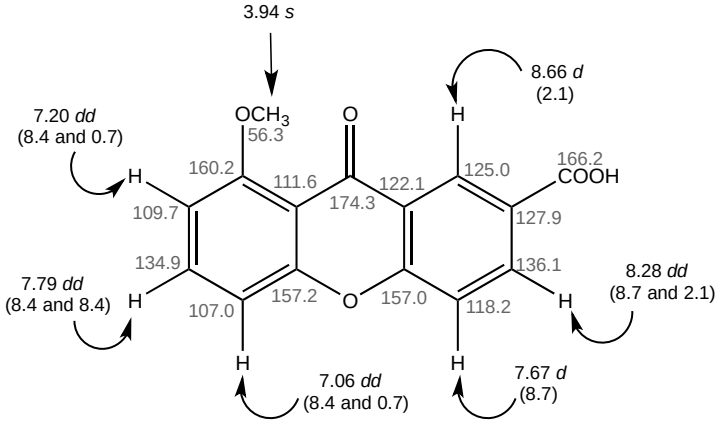
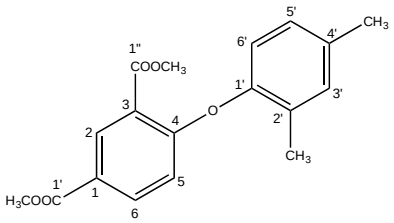


Figure 17: ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-3, **109**).

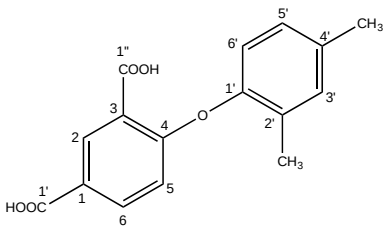
The xanthone 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**109**) showed the following values for HRMS (ESI) m/z: calcd for ($C_{15}H_{10}O_5$ + H): 271.16994, found: 271.06010.

The MS spectra of the compound **117** showed the following peaks and *m/z* values: 284 [M]+. (100), 255 (56), 253 (30), 197 (14), 238 (56), 223 (47), 195 (27), 139 (33), 126 (20), 111 (18), 70 (15), 63 (12).

1.2.5. Structure elucidation of dimethyl 4-(2,4-dimethylphenoxy)isophthalate (119) and dimethyl 4-(2,4-dimethylphenoxy)isophthalic acid (120)



(**119**)



(**120**)

Dimethyl 4-(2,4-dimethylphenoxy)isophthalate (**119**) resulted from the Ullmann diaryl ether coupling of dimethyl 4-bromoisophthalate (**112**) and 2,4-dimethylphenol (**118**). The compound 4-(2,4-dimethylphenoxy)isophthalic acid (**120**) resulted from the hydrolysis

of the methyl esters of compound **119**. The data obtained from IR, ^{1}H and ^{13}C NMR allowed the structure elucidation of compounds **119** and **120**.

Comparing the IR spectrum of the compound **119** (**Table 7**) with the spectrum of its precursor (**112**) (**Table 3**) showed the presence of the absortion bands corresponding to the C=O and to aromatic C=C in both spectra. The main difference important to highlight is the presence of the absorption band C-O-C corresponding to the ether bond at (1259 cm^{-1}) the IR spectra of both compounds (**112** and **119**).

Comparing the IR spectra of compounds **119** and **120** (**Table 7**) it can be seen the presence of the absorption bands corresponding to the C=C aromatic ring bond and to C-O-C ether bond in both spectra, with IR frequencies not significantly different. The presence of a broad absorption bands at 2923 cm^{-1} corresponding to the O-H carboxyl acid bond, on spectrum of compound **120** confirmed that the reaction occurred successfully. Moreover, the shift of the band at 1717 cm^{-1} (C=O of $COOCH_3$) to 1613 cm^{-1} (C=O of COOH) confirmed that occurred transformation from $COOCH_3$ to COOH.

Table 7: IR data of dimethyl 4-(2,4-dimethylphenoxy)isophthalate (**119**) and dimethyl 4-(2,4-dimethylphenoxy)isophthalic acid (**120**).

Bond	υ (cm^{-1})	
	(**119**)	(**120**)
C=O	1717	1613
C=C (aromatic)	1436	1491 and 1604
C-O-C (ether)	1259	1257
O-H	---	2923

The ^{1}H and ^{13}C NMR spectra data obtained for compounds **119** (**Figure 18**) and **120** (**Figure 19**) are consistent to the proposed structure for these compounds, and allowed not only to confirm the success of the transformation, but also to structurally elucidate them.

The ^{1}H NMR spectrum of compound **119**, compared to the spectrum of its precursor (**112**) (**Figure 11**), presented new signals such as two singlets at δ 2.29 ppm and 2.07 ppm, with the integration for three protons each, corresponding to the two Ar-CH_3, confirming the desired transformation.

Compounds **119** and **120** showed similar NMR profiles with respect to the aromatic protons with similar chemical shifts and coupling constants. The most significant difference in the ^{1}H NMR spectra is the absence in compound **120** spectrum of two

singlets at δ 3.84 ppm, with the integration for three protons each, corresponding to the protons of the two COOC$\underline{H}_3$ methyl groups of compound **120**. This difference confirmed the success of the desired transformation.

The ^{13}C NMR spectrum of compound **119** compared to the spectrum of precursor (**112**) (**Figure 11**), presented new spectrum signals at δ 20.4 and 21.0 ppm, corresponding to the carbons of Ar-$\underline{C}H_3$ groups, and associated to the signals of aromatic carbons, confirmed the desired transformation.

The most significant differences between the ^{13}C NMR spectra of the compounds **119** and **120** are the shift of carbon signals from δ 166.1 and 166.0 ppm ($\underline{C}OOCH_3$) to 166.3 and 166.2 ppm ($\underline{C}OOH$), and the absence, in the spectrum of compound **120**, of two signals at δ 52.3 and 52.2 ppm, corresponding to the carbons of COO$\underline{C}H_3$ methyl groups. The referred differences confirmed the success of the chemical modification.

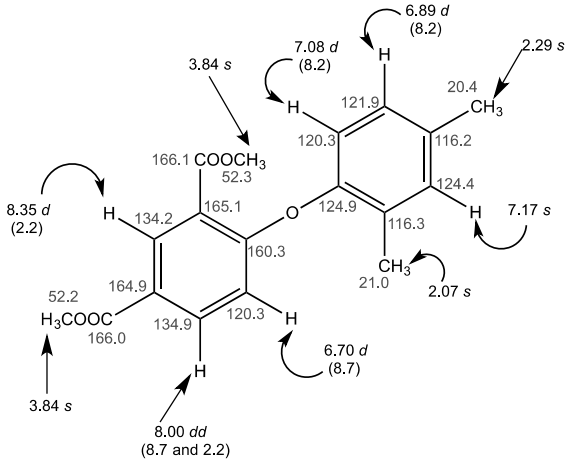


Figure 18: ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of dimethyl 4-(2,4-dimethylphenoxy)isophthalate (**119**).

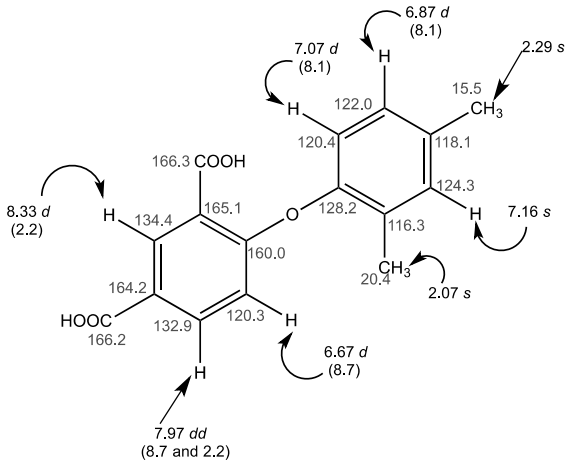
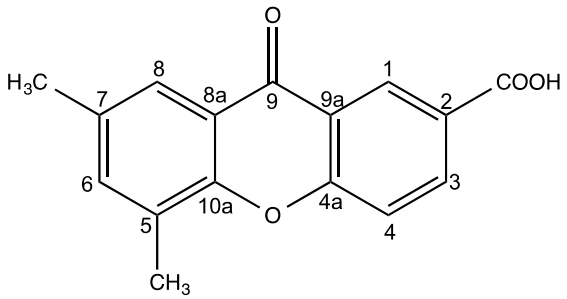


Figure 19: ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of dimethyl 4-(2,4-dimethylphenoxy)isophthalate acid (**120**)).

1.2.6. Structure elucidation of 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, 110)

The 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, **110**) resulted from an intramolecular acylation reaction of the dimethyl 4-(2,4-dimethylphenoxy)isophthalic acid (**120**). The IR, ^{1}H and ^{13}C NMR data allowed the structure elucidation of XCar-5 (**110**).



(**110**)

The IR spectrum of XCar-5 (**110**) (**Table 8**) showed the presence of the absorption bands corresponding to the C=O and aromatic C=C bonds associated with the xanthonic scaffold. It is important to point out the presence of the absorption bands at 680 and 768 cm^{-1} corresponding to the Ar-CH_3 methyl groups.

Table 8: IR data of 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, **110**).

Bond	υ (cm^{-1})
	(**110**)
O-H	2920
C=O (ketone)	1667
C=O (carboxylic acid)	1611
C=C (aromatic)	1475 and 1421
Ar-CH_3	680 and 768

The ^{1}H and ^{13}C NMR spectra data of compound **110** (**Figure 20**) is consistent to the proposed structure for this compound, and allowed not only to confirm the success of the transformation, but also to structurally elucidate it.

The ^{1}H NMR spectrum of compound **110** showed signals corresponding to the five

aromatic protons of the xanthonic scaffold and to the six protons of the two methyl groups.

Comparing of ^{13}C NMR spectra of the compound **110** and its precursor (**120**) (**Figure 19**) it is important to point out the presence of a new signal at δ 176.8 ppm corresponding to the carbonyl group, characteristic of the xanthonic scaffold, confirming the desired transformation. The carbon shifts were assigned with the help of HSQC and HMBC techniques.

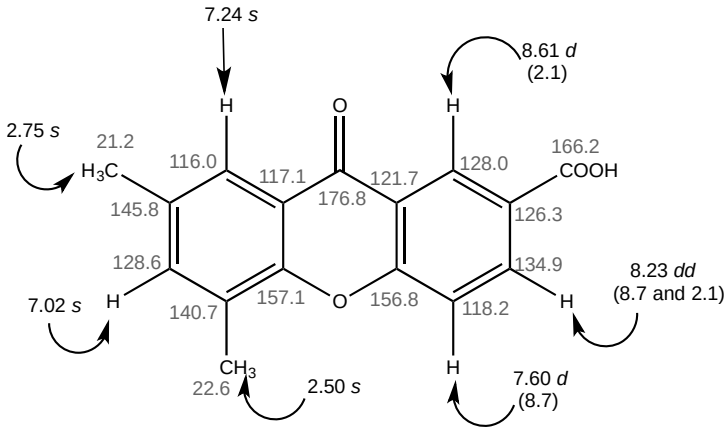
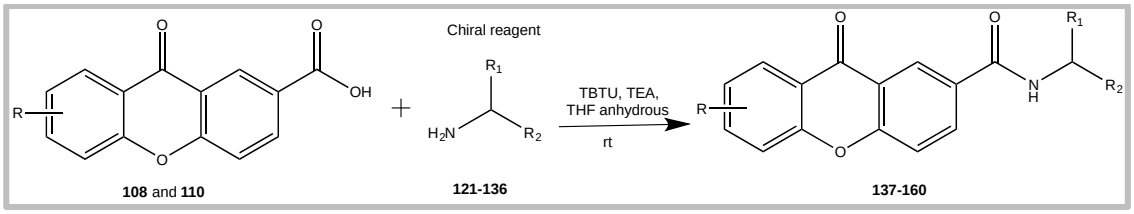


Figure 20: ^{1}H NMR (DMSO-D_6, 300.13 MHz) and ^{13}C NMR (DMSO-D_6, 75.47 MHz) of 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, **110**).

1.3. Synthesis of chiral xanthone derivatives

Twenty four new chiral derivatives of xanthones (CDXs) were synthesized by coupling reaction of the carboxyxanthones 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, **108**) and 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-5, **110**) with a variety of a commercially available enantiomerically pure building blocks (**121-136**) through an amide bond (**Scheme 3**).

The chosen enantiomerically pure building blocks included the amines and amino alcohols used in a previous work,[30] which CDXs afforded the most interesting results considering not only the growth inhibitory effects on the human tumor cell lines tested but also enantioselectivity. Other analogue amines were also selected. Regardless the enantiomerically pure building block, the primary amine was the reactive group for the amide formation.



Scheme 3: General scheme of coupling reaction of carboxyxanthone derivatives (**108** and **110**) with enantiomerically pure building blocks (**121-136**).

Therefore, eight new CDXs, namely, (*S*)-XEA-3-MET (**137**), (*R*)-XEA-3-MET (**138**), (*S*)-XEA-4-MET (**139**), (*R*)-XEA-4-MET (**140**), (*S*)-XEA-4-CLO (**141**), (*R*)-XEA-4-CLO (**142**), (*S*)-XEA-4-FLU (**143**), (*R*)-XEA-4-FLU (**144**), (**Table 9** and **Table 25** of **Appendix**) were synthesized by coupling the XCar-2 (**108**) with (*S*)-3-methoxy-α-methylbenzylamine, (*R*)-3-methoxy-α-methylbenzylamine, (*S*)-4-methoxy-α-methylbenzylamine, (*R*)-4-methoxy-α-methylbenzylamine, (*S*)-4-chloro-α-mehtylbenzylamine, (*R*)-4-chloro-α-mehtylbenzylamine, (*S*)-4-fluoro-α-methylbenzylamine and (*R*)-4-fluoro-α-methylbenzylamine, respectively.

Moreover, twelve new CDXs, namely, (*S*)-XEA5-3-MET (**145**), (*R*)-XEA5-3-MET (**146**), (*S*)-XEA5-4-MET (**147**), (*R*)-XEA5-4-MET (**148**), (*S*)-XEA5-4-CLO (**149**), (*R*)-XEA5-4-CLO (**150**), (*S*)-XEA5-4-FLU (**151**), (*R*)-XEA5-4-FLU (**152**), (*S*)-XEA5 (**153**), (*R*)-XEA5 (**154**), (*S*)-XEA5-DES (**155**), (*R*)-XEA5-DES (**156**), (**Table 9** and **Table 25** of **Appendix**) were synthesized by coupling the XCar-5 (**110**) with both enantiomers of the same four amines mentioned above as well as both enantiomers of α-dimethylbenzylamine and α-methylbenzylamine.

Furthermore, with the same xanthonic chemical substrate (XCar-5, **110**) four new CDXs were synthesized, namely (*S*,*S*)-X2ADF5 (**157**), (*R*,*R*)-X2ADF5 (**158**), (*S*,*R*)-X2ADF5 (**159**) and (*R*,*S*)-X2ADF5 (**160**) (**Table 9** and **Table 25** of **Appendix**) using the four isomers of the aminoalcohol 2-amino-1,2-diphenylethanol as building blocks.

The activation of the carboxylic acid group attached to the xanthonic scaffold of both xanthonic chemical substrates (**108** and **110**) was carried out with *O*-(benzotriazol-1-yl-)-*N*,*N*,*N'*,*N'*-tetramethylluronium tetrafluoroborate (TBTU) (**Figure 21**), in the presence of a catalytic amount of triethylamine (TEA) in dry tetrahydrofuran (THF) (**Scheme 3**). Despite a high number of coupling reagents described in the literature, TBTU is one of the most used due to its higher efficiency and lower tendency towards racemization.[82]

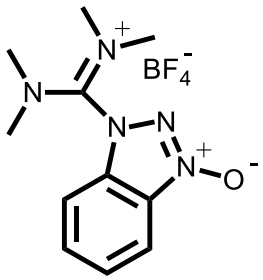


Figure 21: The chemical structure of TBTU.

Table 9 and **Table 10** summarize the results obtained with the coupling reactions for the synthesis of CDXs by using XCar-2 (**108**) and XCar-5 (**110**), respectively, as chemical substrates.

All the reactions were carried out at room temperature and, as demonstrated in **Table 9** and **Table 10**, showed good yields (above 95%) and short reaction times (20 min – 1.5 h). As expected, the syntheses worked equally well with both carboxyxanthones affording the desired amides. The purification procedures were easy, involving liquid-liquid extraction followed by crystallization.

Table 9: Results obtained for the synthesis of CDXs using XCar-2 (**108**) as chemical substrate.

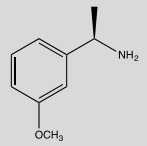
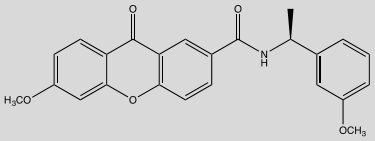
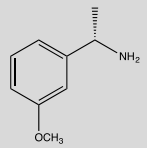
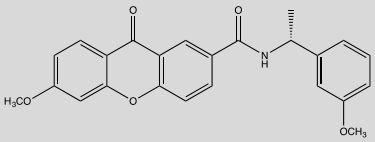
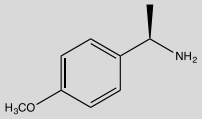
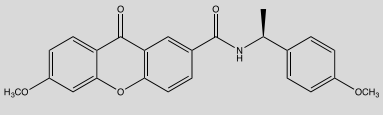
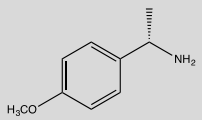
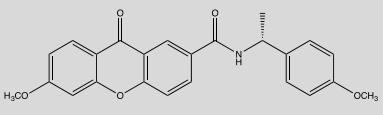
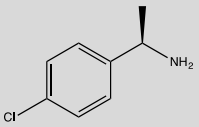
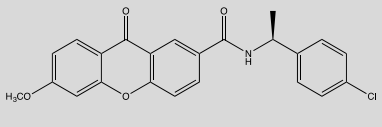
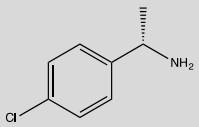
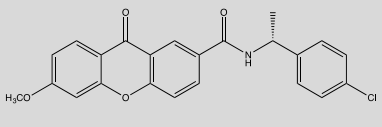
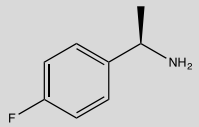
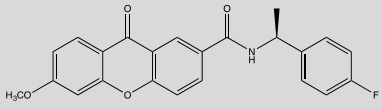
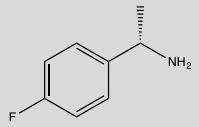
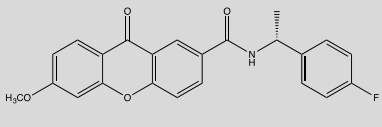
Enantiomerally pure building block	Product (CDX)	Yield	Reaction time	$[\alpha]_D^{25°C}$ (c)*
 (**121**)	 **(*S*)-XEA-3-MET (137)**	96.8%	30 min	-36.4° (5.5×10^{-3})
 (**122**)	 **(*R*)-XEA-3-MET (138)**	97.2%	30 min	+39.2° (5.5×10^{-3})
 (**123**)	 **(*S*)-XEA-4-MET (139)**	98.4%	30 min	+26.5° (5.6×10^{-3})
 (**124**)	 **(*R*)-XEA-4-MET (140)**	96.7%	30 min	-28.5° (5.6×10^{-3})

Table 9 : Continuation

 (**125**)	 **(*S*)-XEA-4-CLO (141)**	98.9%	20 min	+136.7° (5.3×10^{-3})*1
 (**126**)	 **(*R*)-XEA-4-CLO (142)**	97.9%	20 min	-135.9° (5.3×10^{-3})*1
 (**127**)	 **(*S*)-XEA-4-FLU (143)**	96.4%	20 min	+45.5° (5.7×10^{-3})
 **(128)**	 **(*R*)-XEA-4-FLU (144)**	97.1%	20 min	-52.5° (5.7×10^{-3})

* Specific rotation in acetone except *1(in chloroform) with c = concentration in g/mL.

Table 10: Results obtained for the synthesis of CDXs using XCar-5 (**110**) as chemical substrate.

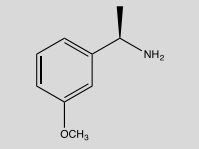
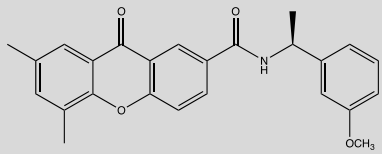
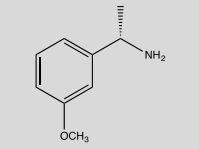
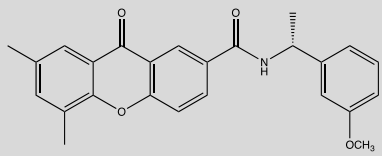
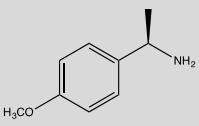
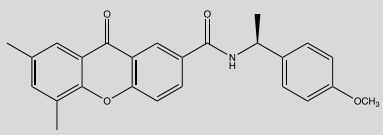
Enantiomeracally pure building block	Product (CDX)	Yield	Reaction time	$[\alpha]_D^{25°C}$ (c)*
 **(121)**	 **(*S*)-XEA5-3-MET (145)**	97.2%	1 h	+183.7° (3.7×10^{-3})
 **(122)**	 **(*R*)-XEA5-3-MET (146)**	96.2%	1 h	-173.5° (3.7×10^{-3})
 **(123)**	 **(*S*)-XEA5-4-MET (147)**	95.8%	1 h	+179.3° (4.3×10^{-3})

Table 10: Continuation

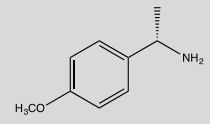
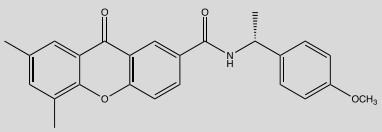
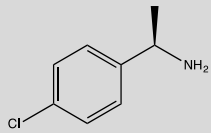
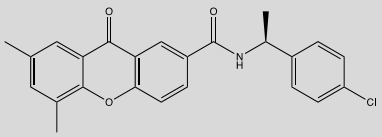
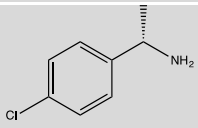
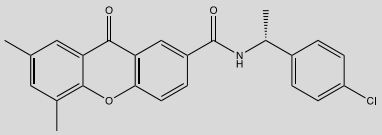
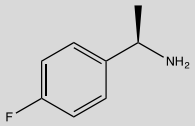
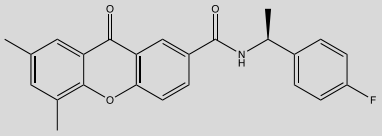
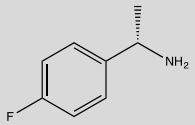
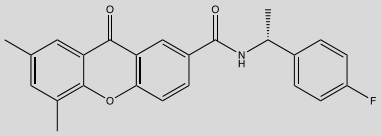
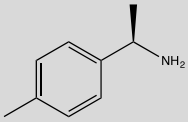
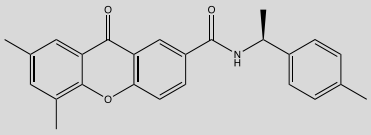
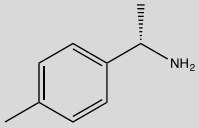
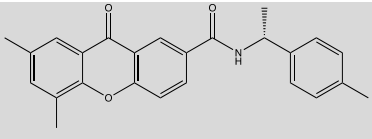
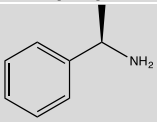
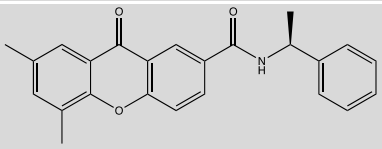
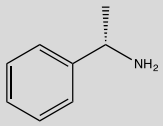
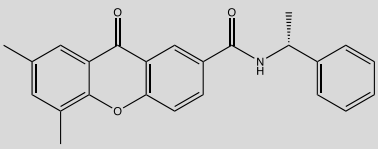
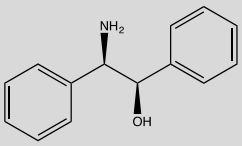
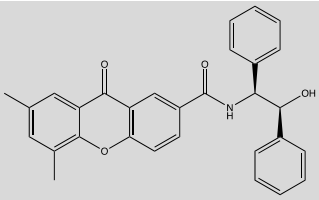
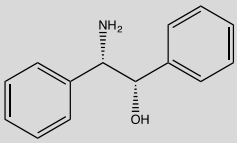
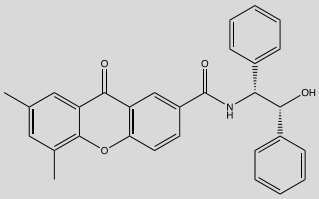
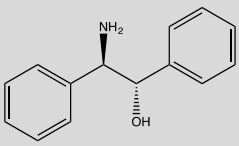
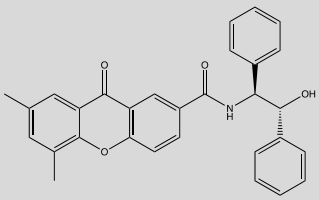
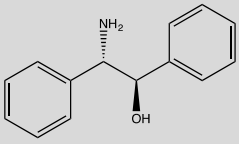
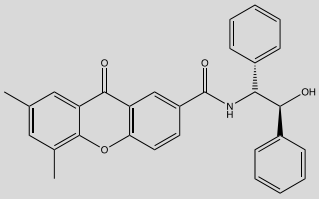
 **(124)**	 **(*R*)-XEA5-4-MET (148)**	98.7%	1 h	-177.6° (4.4×10^{-3})
 **(125)**	 **(*S*)-XEA5-4-CLO (149)**	96.8%	1 h	+204.2° (4.7×10^{-3})
 **(126)**	 **(*R*)-XEA5-4-CLO (150)**	97.9%	1 h	-203.4° (4.7×10^{-3})
 **(127)**	 **(*S*)-XEA5-4-FLU (151)**	95.8%	1.5 h	+167.4° (4.3×10^{-3})
 **(128)**	 **(*R*)-XEA5-4-FLU (152)**	96.3%	1.5 h	-166.7° (4.2×10^{-3})
 **(129)**	 **(*S*)-XEA5 (153)**	97.9%	1.5 h	+130.7° (3.9×10^{-3})
 **(130)**	 **(*R*)-XEA5 (154)**	99.1%	1 h	-125.8° (3.9×10^{-3})
 **(131)**	 **(*S*)-XEA5-DES (155)**	98.9%	1 h	+132.5° (4.0×10^{-3})

Table 10: Continuation

 **(132)**	 **(*R*)-XEA5-DES (156)**	97.7%	1 h	-131.5 (4.0×10^{-3})
 **(133)**	 **(*S*,*S*)-X2ADF5 (157)**	97.3%	1.5 h	-30.2° (4.3×10^{-3})
 **(134)**	 **(*R*,*R*)-X2ADF5 (158)**	96.9%	1.5 h	+52.8° (4.3×10^{-3})
 **(135)**	 **(*S*,*R*)-X2ADF5 (159)**	97.0%	1.5 h	+32.2° (4.3×10^{-3})
 **(136)**	 **(*R*,*S*)-X2ADF5 (160)**	97.5%	1.5 h	-39.0° (4.3×10^{-3})

* Specific rotation in chloroform with c = concentration in g/mL.

1.4. Structure elucidation of chiral derivatives of xanthones

The IR, ^{1}H and ^{13}C NMR allowed the structure elucidation of all CDXs.

The data obtained by analysis of IR spectra of CDXs obtained from XCar-2 (**108**) and XCar-5 (**110**) are listed on **Table 11** and **Table 12**, respectively.

Table 11: IR data of CDXs obtained from XCar-2 (**108**).

	υ (cm^{-1})					
	C=O ketone	**C-H aromatic**	**C=C aromatic**	**Ar-OCH_3**	**C=O amide**	**N-H**
(*S*)-XEA-3-MET (**137**)	1663	833	1440, 1469, 1482, 1533	1270	1631	3319
(*R*)-XEA-3-MET (**138**)	1666	833	1440, 1470, 1482, 1533	1271	1633	3319
(*S*)-XEA-4-MET (**139**)	1655	831	1443, 1478, 1513, 1539	1272	1629	3318
(*R*)-XEA-4-MET (**140**)	1658	831	1443, 1479, 1537	1272	1636	3319
(*S*)-XEA-4-CLO (**141**)	1651	826	1445,1480, 1540, 1589	1275	1635	3290
(*R*)-XEA-4-CLO (**142**)	1652	827	1445,1481, 1540, 1590	1274	1635	3296
(*S*)-XEA-4-FLU (**143**)	1665	833	1443, 1476, 1511	1274	1634	3316
(*R*)-XEA-4-FLU (**144**)	1665	834	1443, 1539	1274	1630	3317

Table 12: IR data of CDXs obtained from XCar-5 (**110**).

	υ (cm^{-1})					
	C=O ketone	**C-H aromatic**	**C=C aromatic**	**O-H**	**C=O amide**	**N-H**
(*S*)-XEA5-3-MET (**145**)	1664	791, 837	1473, 1540 1606	---	1633	3289
(*R*)-XEA5-3-MET (**146**)	1664	792, 837	1470, 1540 1605	---	1634	3297
(*S*)-XEA5-4-MET (**147**)	1662	792, 829	1466, 1515, 1539, 1614	---	1634	3303
(*R*)-XEA5-4-MET (**148**)	1662	793, 829	1467, 1540, 1610	---	1633	3300
(*S*)-XEA5-4-CLO (**149**)	1667	791, 825	1474, 1536, 1611	---	1629	3297
(*R*)-XEA5-4-CLO (**150**)	1666	791, 828	1470, 1540, 1611	---	1631	3299
(*S*)-XEA5-4-FLU (**151**)	1673	790, 824	1473, 1555, 1609	---	1637	3304
(*R*)-XEA5-4-FLU (**152**)	1672	792, 829	1463, 1555, 1608	---	1637	3303

Table 12: Continuation

(*S*)-XEA5 (**153**)	1667	790, 834	1466, 1547, 1609	---	1632	3298
(*R*)-XEA5 (**154**)	1667	791, 832	1466, 1542, 1612	---	1634	3300
(*S*)-XEA5-DES (**155**)	1666	791, 833	1475, 1536, 1611	---	1630	3303
(*R*)-XEA5-DES (**156**)	1665	791, 837	1475, 1536, 1612	---	1632	3304
(*S,S*)-X2ADF5 (**157**)	1669	701, 792	1475, 1529, 1613	3421	1634	3320
(*R,R*)-X2ADF5 (**158**)	1668	702, 801	1475, 1489, 1533	3389	1635	3302
(*S,R*)-X2ADF5 (**159**)	1673	701, 755	1474, 1533	3385	1635	3311
(*R,S*)-X2ADF5 (**160**)	1674	700, 754	1474, 154-0, 1614	3418	1636	3311

The IR spectra of all CDXs (**Table 11** and **Table 12**) showed the presence of absorption bands corresponding to the C=O relative the ketone bond, at 1651-1674 cm^{-1} and to aromatic C=C at 1440-1614 cm^{-1}, associated with the xanthonic scaffold. Moreover, the presence of a band at 1270-1275 cm^{-1} corresponding to the Ar-OCH_3 bond was observed for all the CDXs obtained from XCar-2 (**108**) (**Table 11**). For the CDXs obtained by coupling XCar-5 (**110**) with chiral aminoalcohols a broad band at 3385-3421 cm^{-1}, corresponding to O-H bond of the alcohol was also present.

Comparing the IR spectra of xanthonic chemical substrates, namely XCar-2 (**108**) (**Table 5**) and XCar-5 (**110**) (**Table 8**) and CDXs the main changes that are important to highlighted are the presence of strong bands corresponding to N-H around 3300 cm^{-1} and C=O of amine around 1630 cm^{-1},

The ^{1}H and ^{13}C NMR data of CDXs obtained from XCar-2 (**108**) are reported in **Table 13** and **Table 14**, respectively.

The ^{1}H NMR data of CDXs obtained by coupling XCar-5 (**110**) with amines are shown in **Table 15** and **Table 16**, and with aminoalcohols in **Table 17**.

Table 18 and **Table 19** presented the ^{13}C NMR data of CDXs obtained by coupling XCar-5 (**110**) with amines; and **Table 20** with aminoalcohols.

The ^{1}H NMR and the ^{13}C NMR spectra of all CDXs showed chemical shifts corresponding to the aromatic protons and carbons of the xanthonic scaffold.

The more significant differences of ^{1}H NMR and the ^{13}C NMR spectra of all CDXs,

comparing with the spectrum of their precursors, namely XCar-2 (**108**) (**Figure 14**) and XCar-5 (**110**) (**Figure 20**) correspond to the presence of new spectral signals that confirm the success of the coupling reactions. For example, it is important to point out the presence in ^{1}H NMR spectra of the chemical shift around 6.60 ppm corresponding to the proton of amide group, and the presence in ^{13}C NMR spectra of the chemical shift around 165.5 ppm corresponding to the carbonyl of amide group.

For compounds (**137**, **147**) were provided spectral data from HSQC and HMBC experiments to clarify the structural elucidation of these compounds.

Table 13: ^{1}H NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, and J in Hz, for CDXs (**137-144**) obtained from XCar-2. (**108**).

	(*S*)-XEA-3-MET (137)	**(*R*)-XEA-3-MET (138)**	**(*S*)-XEA-4-MET (139)**	**(*R*)-XEA-4-MET (140)**	**(*S*)-XEA-4-CLO (141)**	**(*R*)-XEA-4-CLO (142)**	**(*S*)-XEA-4-FLU (143)**	**(*R*)-XEA-4-FLU (144)**
H1	8.58 (*d*, J=2.2)	8.58 (*d*, J=2.2)	8.56 (*d*, J=2.3)	8.56 (*d*, J=2.3)	8.59 (*d*, J=2.2)	8.59 (*d*, J=2.2)	8.57 (*d*, J=2.3)	8.57 (*d*, J=2.3)
H3	8.32 (*dd*, J=2.3, 8.8)	8.32 (*dd*, J=2.3, 8.8)	8.32 (*dd*, J=2.3, 8.8)	8.32 (*dd*, J=2.3, 8.8)	8.30 (*dd*, J=2.3, 8.8)	8.30 (*dd*, J=2.3, 8.8)	8.32 (*dd*, J=2.3, 8.8)	8.32 (*dd*, J=2.3, 8.8)
H4	7.54 (*d*, J=8.8)	7.54 (*d*, J=8.8)	7.54 (*d*, J=8.8)	7.54 (*d*, J=8.8)	7.53 (*d*, J=8.8)	7.53 (*d*, J=8.8)	7.55 (*d*, J=8.8)	7.55 (*d*, J=8.8)
H5	6.93 (*d*, J=2.3)	6.93 (*d*, J=2.3)	6.93 (*d*, J=2.7)	6.93 (*d*, J=2.7)	6.91 (*d*, J=2.3)	6.91 (*d*, J=2.3)	6.93 (*d*, J=2.3)	6.93 (*d*, J=2.3)
H7	6.97 (*dd*, J=2.3, 8.9)	6.97 (*dd*, J=2.3, 8.9)	6.99 (*dd*, J=2.4, 8.9)	6.99 (*dd*, J=2.4, 8.9)	6.98 (*dd*, J=2.4, 8.9)	6.98 (*dd*, J=2.4, 8.9)	6.99 (*dd*, J=2.3, 8.9)	6.99 (*dd*, J=2.3, 8.9)
H8	8.26 (*d*, J=8.9)	8.26 (*d*, J=8.9)	8.26 (*d*, J=8.9)	8.26 (*d*, J=8.9)	8.24 (*d*, J=8.9)	8.24 (*d*, J=8.9)	8.26 (*d*, J=8.9)	8.26 (*d*, J=8.9)
NH	6.59 (*d,* J=7.8)	6.58 (*d*, J=7.7)	6.59 (*d,* J=7.8)	6.56 (*d,* J=7.8)	6.71 (*d,* J=7.4)	6.67 (*d,* J=7.4)	6.58 (*d,* J=7.4)	6.58 (*d,* J=7.4)
H1'	5.37 - 5.28 (*m*)	5.37 - 5.28 (*m*)	5.37 - 5.28 (*m*)	5.37 - 5.28 (*m*)	5.37 - 5.28 (*m*)	5.37 - 5.28 (*m*)	5.37-5.28 (*m*)	5.37-5.28 (*m*)
H2'	1.64 (*d,* J=6.9)	1.64 (*d,* J=6.9)	1.63 (*d,* J=7.0)	1.63 (*d,* J=7.0)	1.62 (*d,* J=7.0)	1.62 (*d,* J=7.0)	1.63 (*d,* J=7.0)	1.63 (*d,* J=7.0)
H2''	7.30 (*d,* J=7.9)	7.30 (*d,* J=7.9)	7.35 (*d,* J=8.5)	7.35 (*d,* J=8.5)	7.36 - 7.26 (*m*)	7.36 - 7.26 (*m*)	7.41 - 7.37 (*m*)	7.41 - 7.37 (*m*)
H3''	-	-	6.89 (*d,* J=2.1)	6.89 (*d,* J=2.1)	7.36 - 7.26 (*m*)	7.36-7.26 (*m*)	7.08 - 7.03 (*m*)	7.08 - 7.03 (*m*)
H4''	6.83 (*ddd,* J=0.7, 2.5, 8.0)	6.83 (*ddd,* J=0.7, 2.5, 8.0)	-	-	-	-	-	-
H5''	7.00 – 6.98 (*m*)	7.00 – 6.98 (*m*)	6.89 (*d,* J=2.1)	6.89 (*d,* J=2.1)	7.36 - 7.26 (*m*)	7.36 - 7.26 (*m*)	7.08 - 7.03 (*m*)	7.08 - 7.03 (*m*)
H6''	7.00 – 6.98 (*m*)	7.00 – 6.98 (*m*)	7.35 (*d,* J=8.5)	7.35 (*d,* J=8.5)	7.36 - 7.26 (*m*)	7.36 - 7.26 (*m*)	7.41 - 7.37 (*m*)	7.41 - 7.37 (*m*)
OCH_3 xanthone	3.96 (*s*)	3.96 (*s*)	3.96 (*s*)	3.96 (*s*)	3.95 (*s*)	3.95 (*s*)	3.96 (*s*)	3.96 (*s*)
OCH_3 aromatic amine	3.82 (*s*)	3.82 (*s*)	3.81 (*s*)	3.81 (*s*)	-	-	-	-

Table 14: ^{13}C NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, for CDXs (**137-144**) obtained from XCar-2 (**108**).

	(*S*)-XEA-3-MET (**137**)	(*R*)-XEA-3-MET (**138**)	(*S*)-XEA-4-MET (**139**)	(*R*)-XEA-4-MET (**140**)	(*S*)-XEA-4-CLO (**141**)	(*R*)-XEA-4-CLO (**142**)	(*S*)-XEA-4-FLU (**143**)	(*R*)-XEA-4-FLU (**144**)
C1	124.0	124.0	124.0	124.0	124.0	124.0	123.9	123.9
C2	129.8	129.9	130.0	130.1	129.8	129.8	129.8	129.8
C3	134.3	134.3	134.3	134.3	134.3	134.3	134.4	134.3
C4	118.5	118.6	118.5	118.5	118.6	118.6	118.7	118.6
C4a	157.9	157.9	157.9	157.9	157.9	157.9	157.9	157.9
C5	100.4	100.4	100.3	100.3	100.3	100.3	100.4	100.3
C6	164.8	164.6	164.8	164.8	165.0	164.9	164.8	164.8
C7	113.8	113.8	113.8	113.8	113.8	113.8	113.9	113.8
C8	128.4	128.4	128.3	128.3	128.3	128.3	128.4	128.3
C8a	115.5	115.5	115.5	115.5	115.5	115.5	115.6	115.6
C9	175.9	175.9	175.9	175.9	175.9	175.9	175.9	175.9
C9a	121.1	121.1	121.1	121.0	121.0	121.0	121.0	121.0
C10a	158.0	158.0	158.0	158.0	158.0	158.0	158.0	158.0
C=O amide	165.5	165.5	165.5	165.6	165.5	165.5	165.5	165.5
C1'	49.6	49.6	49.0	49.0	49.0	49.0	48.9	48.9
C2' (CH_3)	21.8	21.8	21.6	21.6	21.8	21.8	21.8	21.8
C1''	144.5	144.5	135.0	135.0	133.1	133.1	138.7	138.7
C2''	130.0	130.0	127.5	127.5	127.6	127.6	127.9	127.9
C3''	159.9	159.9	114.1	114.1	117.8	117.8	115.5	115.5
C4''	112.7	112.7	159.0	159.0	141.6	141.6	161.1	161.1
C5''	112.3	112.3	114.1	114.1	117.8	117.8	115.5	115.5
C6''	118.6	118.6	127.5	127.5	127.6	127.6	127.9	127.9
O$\underline{C}H_3$ xanthone	55.9	55.9	55.9	55.9	55.9	55.9	55.9	55.9
O$\underline{C}H_3$ aromatic amine	55.3	55.3	55.3	55.3	-	-	-	-

Table 15: ^{1}H NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, and J in Hz, for CDXs (**145-150**) obtained from XCar-5. (**110**).

	(*S*)-XEA5-3-MET (145)	**(*R*)-XEA5-3-MET (146)**	**(*S*)-XEA5-4-MET (147)**	**(*R*)-XEA5-4-MET (148)**	**(*S*)-XEA5-4-CLO (149)**	**(*R*)-XEA5-4-CLO (150)**
H1	8.60 (*d*, J=2.3)	8.59 (*d*, J=2.3)	8.57 (*d*, J=2.3)	8.57 (*d*, J=2.3)	8.60 (*d*, J=2.3)	8.60 (*d*, J=2.3)
H3	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)
H4	7.59 (*d*, J=8.8)	7.59 (*d*, J=8.8)	7.59 (*d*, J=8.8)	7.59 (*d*, J=8.8)	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)
H6	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)
H8	7.95 (*d*, J=0.8)	7.95 (*d*, J=0.8)	7.96 (*d*, J=0.8)	7.96 (*d*, J=0.8)	7.95 (*d*, J=0.7)	7.95 (*d*, J=0.7)
NH	6.63 (*d*, J=7.6)	6.63 (*d*, J=7.6)	6.60 (*d*, J=7.5)	6.60 (*d*, J=7.5)	6.65 (*d*, J=7.5)	6.65 (*d*, J=7.5)
H1'	5.37–5.27 (*m*)	5.37–5.27 (*m*)	5.37–5.27 (*m*)	5.37–5.27 (*m*)	5.37–5.27 (*m*)	5.37–5.27 (*m*)
H2' (CH_3)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.63 (*d*, J=6.9)	1.63 (*d*, J=6.9)
H2''	7.27 (*d*, J=7.9)	7.27 (*d*, J=7.9)	7.35 (*d*, J=8.7)	7.35 (*d*, J=8.7)	7.36 (*d*, J=8.8)	7.36 (*d*, J=8.8)
H3''	-	-	6.90 (*d*, J=8.7)	6.90 (*d*, J=8.7)	7.31 (*d*, J=8.8)	7.31 (*d*, J=8.8)
H4''	6.82 (*ddd*, J=8.0, 2.6, 0.8)	6.82 (*ddd*, J=8.0, 2.6, 0.8)	-	-	-	-
H5''	7.02 – 6.96 (*m*)	7.02 – 6.96 (*m*)	6.90 (*d*, J=8.7)	6.90 (*d*, J=8.7)	7.31 (*d*, J=8.8)	7.31 (*d*, J=8.8)
H6''	7.02 – 6.96 (*m*)	7.02 – 6.96 (*m*)	7.35 (*d*, J=8.7)	7.35 (*d*, J=8.7)	7.36 (*d*, J=8.8)	7.36 (*d* J=8.8)
CH_3 (C5 xanthone)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)
CH_3 (C7 xanthone)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)
Ar-OCH_3	3.81 (*s*)	3.81 (*s*)	3.80 (*s*)	3.80 (*s*)	-	-

Table 16: ^{1}H NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, and J in Hz, for CDXs (**151-156**) obtained from from XCar-5 (110).

	(*S*)-XEA5-4-FLU (151)	**(*R*)-XEA5-4-FLU (152)**	**(*S*)-XEA5 (153)**	**(*R*)-XEA5 (154)**	**(*S*)-XEA5-DES (155)**	**(*R*)-XEA5-DES (156)**
H1	8.58 (*d*, J=2.3)	8.58 (*d*, J=2.3)	8.58 (*d*, J=2.3)	8.58 (*d*, J=2.3)	8.59 (*d*, J=2.3)	8.59 (*d*, J=2.3)
H3	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3,8.8)	8.33 (*dd*, J=2.3, 8.8)	8.33 (*dd*, J=2.3, 8.8)	8.33 (*dd*, J=2.3, 8.8)	8.33 (*dd*, J=2.3, 8.8)
H4	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)	7.60 (*d*, J=8.8)
H6	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.8)	7.43 (*d*, J=0.9)	7.43 (*d*, J=0.9)	7.43-7.27 (*m*)	7.43-7.27 (*m*)
H8	7.98 (*d*, J=0.7)	7.98 (*d*, J=0.7)	7.97 (*d*, J=0.9)	7.97 (*d*, J=0.9)	7.97 (*d*, J=0.9)	7.97 (*d*, J=0.9)
NH	6.58 (*d*, J=7.5)	6.68 (*d*, J=7.5)	6.60 (*d*, J=7.6)	6.60 (*d*, J=7.6)	6.65 (*d*, J=7.6)	6.65 (*d*, J=7.6)
H1'	5.38–5.28 (*m*)	5.38–5.28 (*m*)	5.37–5.27 (*m*)	5.37–5.27 (*m*)	5.41–5.32 (*m*)	5.41–5.32 (*m*)
H2' ($C\underline{H}_3$)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.64 (*d*, J=6.9)	1.66 (*d*, J=6.9)	1.66 (*d*, J=6.9)
H2''	7.41 (*d*, J=8.7)	7.39 (*d*, J=8.7)	7.33 (*d*, J=8.0)	7.33 (*d*, J=8.0)	7.43-7.27 (*m*)	7.43-7.27 (*m*)
H3''	7.07 (*d*, J=8.7)	7.07 (*d*, J=8.7)	7.18 (*d*, J=8.0)	7.18 (*d*, J=8.0)	7.43-7.27 (*m*)	7.43-7.27 (*m*)
H4''	-	-	-	-	7.43-7.27 (*m*)	7.43-7.27 (*m*)
H5''	7.07 (*d*, J=8.7)	7.07 (*d*, J=8.7)	7.18 (*d*, J=8.0)	7.18 (*d*, J=8.0)	7.43-7.27 (*m*)	7.43-7.27 (*m*)
H6''	7.41 (*d*, J=8.7)	7.39 (*d*, J=8.7)	7.33 (*d*, J=8.0)	7.33 (*d*, J=8.0)	7.43-7.27 (*m*)	7.43-7.27 (*m*)
$C\underline{H}_3$ (C5 xanthone)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)	2.54 (*s*)
$C\underline{H}_3$ (C7 xanthone)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)	2.44 (*s*)
Ar-$C\underline{H}_3$	-	-	2.34 (*s*)	2.34 (*s*)	-	-

Table 17: ^{1}H NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, and J in Hz, for CDXs (**157-160**) obtained from from XCar-5 (**110**).

	(*S*,*S*)-X2ADF5 (157)	**(*R*,*R*)-X2ADF5 (158)**	**(*S*,*R*)-X2ADF5 (159)**	**(*R*,*S*)-X2ADF5 (160)**
H1	8.55 (*d*, J=2.1)	8.76 (*d*, J=2.1)	8.64 (*d*, J=2.1)	8.64 (*d*, J=2.1)
H3	8.09 (*dd*, J=2.1, 8.8)	7.76 (*dd*, J=2.1, 8.8)	8.20 (*dd*, J=2.1, 8.8)	8.29 (*dd*, J=2.1, 8.8)
H4	7.68 (*d*, J=8.8)	7.76 (*d*, J=8.8)	7.56 (*d*, J=8.8)	7.59 (*d*, J=8.8)
H6	7.57 (*d*, J=0.8)	7.61 (*d*, J=0.8)	7.42 (*d*, J=0.8)	7.44 (*d*, J=0.8)
H8	7.82 (*d*, J=0.8)	7.86 (*d*, J=0.8)	7.97 (*d*, J=0.8)	7.97 (*d*, J=0.8)
NH	9.01 (*d*, J=8.3)	9.18 (*d*, J=8.3)	9.18 (*d*, J=8.3)	9.18 (*d*, J=8.3)
H1'	5.21 – 5.15 (*m*)	5.26 – 5.21 (*m*)	5.46 – 5.42 (*m*)	5.53 – 5.49 (*m*)
H2' (OH)	4.99 (*d*, J=5.1)	4.99 (*d*, J=5.1)	5.16 (*d*, J=5.1)	5.16 (*d*, J=5.1)
Aromatics (chiral moiety)	7.33 – 7.16 (*m*)	7.32 – 7.14 (*m*)	7.38 – 7.24 (*m*)	7.38 – 7.22 (*m*)
C$\underline{H}_3$ (C5 xanthone)	2.51 (*s*)	2.51 (*s*)	2.53 (*s*)	2.53 (*s*)
C$\underline{H}_3$ (C7 xanthone)	2.48 (*s*)	2.48 (*s*)	2.45 (*s*)	2.45 (*s*)
O$\underline{H}$	5.70 (*d*, J=5.1)	5.70 (*d*, J=5.1)	5.70 (*d*, J=5.1)	5.70 (*d*, J=5.1)

Table 18: ^{13}C NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, for CDXs (**145-150**) obtained from XCar-5 (**110**).

	(*S*)-XEA5-3-MET (**145**)	(*R*)-XEA5-3-MET (**146**)	(*S*)-XEA5-4-MET (**147**)	(*R*)-XEA5-4-MET (**148**)	(*S*)-XEA5-4-CLO (**149**)	(*R*)-XEA5-4-CLO (**150**)
C1	124.2	124.1	124.1	124.0	124.1	124.1
C2	129.8	129.8	129.9	129.9	129.6	129.5
C3	134.5	134.5	134.5	134.5	134.5	134.5
C4	118.9	118.9	118.9	118.9	119.0	119.0
C4a	157.7	157.7	157.7	157.7	157.8	157.8
C5	127.1	127.1	127.1	127.1	127.2	127.2
C6	137.6	137.6	137.6	137.6	137.7	137.7
C7	133.8	133.8	133.7	133.7	133.8	133.8
C8	123.6	123.6	123.6	123.6	123.6	123.6
C8a	121.1	121.1	121.1	121.1	121.1	121.1
C9	177.3	177.3	177.3	177.3	177.3	177.3
C9a	120.6	120.6	120.6	120.6	120.6	120.6
C10a	152.7	152.7	152.7	152.7	152.7	152.7
C=O amide	164.9	164.9	164.8	164.8	165.0	164.9
C1'	49.5	49.5	49.0	49.0	49.0	49.0
C2' (CH_3)	21.7	21.8	21.6	21.6	21.8	21.8
C1''	144.6	144.6	135.0	135.0	133.2	133.2
C2''	112.3	112.3	127.5	127.5	128.8	128.8
C3''	159.8	159.8	114.1	114.1	127.7	127.7
C4''	112.3	112.3	158.9	158.9	141.6	141.6
C5''	118.5	118.5	114.1	114.1	127.7	127.7
C6''	112.7	112.7	127.5	127.5	128.8	128.8
CH_3 (C5 xanthone)	15.7	15.7	15.7	15.7	15.7	15.7
CH_3 (C7 xanthone)	20.8	20.8	20.8	20.8	20.8	20.8
Ar-O$\underline{C}H_3$	55.2	55.2	55.3	55.3	-	-

Table 19: ^{13}C NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, for CDXs (**151-156**) obtained from XCar-5 (**110**).

	(*S*)-XEA5-4-FLU (**151**)	(*R*)-XEA5-4-FLU (**152**)	(*S*)-XEA5 (**153**)	(*R*)-XEA5 (**154**)	(*S*)-XEA5-DES (**155**)	(*R*)-XEA5-DES (**156**)
C1	124.0	124.0	124.1	124.4	124.1	124.1
C2	129.7	129.7	129.9	129.9	129.8	129.8
C3	134.5	134.5	134.5	134.5	134.5	134.5
C4	119.0	119.0	118.9	118.9	118.9	118.9
C4a	157.8	157.8	157.7	157.9	157.7	157.7
C5	127.2	127.2	127.1	127.1	127.1	127.1
C6	137.7	137.7	137.6	137.6	137.6	137.6
C7	133.9	133.9	133.8	133.8	133.8	133.8
C8	123.6	123.6	123.6	123.6	123.6	123.6
C8a	121.1	121.1	121.1	121.1	121.1	121.1
C9	177.3	177.3	177.3	177.3	177.3	177.3
C9a	120.6	120.6	120.6	120.6	120.6	120.6
C10a	152.7	152.7	152.7	152.7	152.7	152.7
C=O amide	165.0	165.0	164.8	164.8	164.9	164.9
C1'	48.9	48.9	49.4	49.4	49.5	49.5
C2' (CH_3)	21.8	21.8	21.1	21.1	21.7	21.7
C1''	135.0	135.0	137.2	137.2	142.9	142.9
C2''	128.0	128.0	129.4	129.4	126.3	126.3
C3''	115.6	115.6	126.2	126.2	128.7	128.8
C4''	138.8	138.8	139.9	139.9	127.5	127.5
C5''	115.6	115.6	126.2	126.2	128.7	128.8
C6''	128.0	128.0	129.4	129.4	126.3	126.3
CH_3 (C5 xanthone)	15.7	15.7	15.7	15.7	15.7	15.7
CH_3 (C7 xanthone)	20.8	20.8	20.8	20.8	20.8	20.8
Ar-C$\underline{H}_3$	-	-	21.7	21.7	-	-

Table 20: ^{13}C NMR ($CDCl_3$ or DMSO-D_6, 300.13 MHZ), chemical shift values, δ, in ppm, for CDXs (**157-160**) obtained from XCar-5 (**110**).

	(*S*,*S*)-X2ADF5 (157)	**(*R*,*R*)-X2ADF5 (158)**	**(*S*,*R*)-X2ADF5 (159)**	**(*R*,*S*)-X2ADF5 (160)**
C1	125.2	125.5	125.5	124.5
C2	130.1	130.2	129.6	129.6
C3	134.0	134.2	134.3	134.4
C4	118.5	118.5	118.9	118.9
C4a	156.9	156.9	157.8	157.8
C5	127.0	127.0	127.1	127.1
C6	137.6	137.6	137.6	137.6
C7	133.5	133.5	133.8	133.8
C8	122.9	122.9	123.6	123.6
C8a	120.6	120.7	121.1	121.1
C9	176.1	176.2	177.2	177.2
C9a	120.1	120.2	120.6	120.7
C10a	152.7	152.7	152.7	152.7
C=O amide	163.8	164.6	166.0	165.6
C1'	59.25	59.25	60.24	59.85
CHOH	74.54	75.55	74.90	74.90
C1''	143.6	143.0	140.7	140.7
C2'' and C6''	127.6	127.7	127.9	128.1
C3'' and C5''	128.4	127.8	127.8	128.2
C4''	126.9	126.8	127.0	127.8
C1'''	141.3	141.2	139.4	139.7
C2''' and C6'''	127.0	127.0	127.8	128.0
C3''' and C5'''	127.7	127.7	127.4	128.2
C4'''	126.7	126.6	126.1	126.5
CH_3 (C5 xanthone)	15.19	15.24	15.72	15.72
CH_3 (C7 xanthone)	20.34	20.36	20.86	20.86

2. Enantiomeric purity

LC has proven to be one of the best methods for the separation and analysis of enantiomers.[108,109] Moreover, the LC using CSPs is the most helpful between the currently analytical methods to determine the enantiomeric purity.[85]

The chiral separation method should take only a short analysis time and preferably use inexpensive and "green" solvents. Herein, we explored the LC enantioseparation of the synthesized CDXs based on a recent study using Pirkle-type CSPs.[86] The (*S*,*S*)-Whelk-O1 CSP (**Figure 22**) was chosen since it demonstrated a very good enantioresolution performance for CDXs possessing an aromatic moiety linked to the stereogenic center with excellent separation (α) and resolution (Rs) factors, under polar-organic conditions.[86]

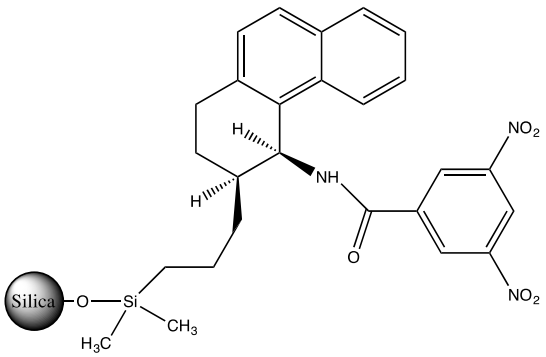


Figure 22: The chemical structure of (*S*,*S*)-Whelk-O1 CSP.

The mixture of two polar-organic solvents, specifically acetonitrile (ACN) and methanol (MeOH) (50:50 *v/v*), as mobile phase has been tested in our group and showed excellent results with outstanding enantioselectivity, resolution and shorter retention time. The polar-organic conditions proved to be a best alternative to the normal-phase conditions.[86]

Consequently, and based on described work,[86] enantiomeric mixtures of the synthesized CDXs were prepared and separated using the following chromatographic conditions: (*S*,*S*)-Whelk-O1 CSP, MeOH:ACN (50:50 *v/v*) as mobile phase, flow rate at 1.0 mL/min and U.V detection at 254nm. The chromatographic data obtained for all the enantiomeric mixtures of CDXs is shown in **Table 21.**

Table 21: Chromatographic results obtained on (*S*,*S*)-Whelk-O1 CSP, under polar organic elution condition, for the enantiomeric mixtures of synthesized CDXs.

Enantiomeric mixtures of CDXs	k_1	k_2	α	R_S
XEA-3-MET	0.61	3.20	5.27	10.55
XEA-4-MET	0.67	3.32	4.92	15.09
XEA-4-FLU	0.49	2.01	4.07	11.46
XEA-4-CLO	0.66	3.91	5.91	16.59
X2ADF5 – RR,SS	0.48	0.82	1.69	2.42
X2ADF5 -RS,SR	0.44	0.68	1.53	2.12
XEA5	0.64	3.23	5.01	11.55
XEA5-DES	0.61	2.58	4.22	11.73
XEA5-3-MET	0.66	3.96	5.97	16.84
XEA5-4-MET	0.79	4.65	5.84	17.20
XEA5-4-FLU	0.54	2.54	4.65	11.87
XEA5-4-CLO	0.67	4.20	6.25	16.22

Mobile phase conditions: MeOH:ACN (50:50 *v/v*), flow rate 1.0 mL/min. k = retention factor, α = separation factor, Rs = resolution factor.

All enantiomeric mixtures of CDXs were enantioseparated with excellent enantioselectivity on (*S,S*)-Whelk-O1 CSP, with α ranging from 1.53 to 6.25 and resolutions ranging from 2.12 to 17.20. The (*R*)-enantiomer of all enantiomeric pairs of CDXs was the first to elute in the tested conditions.

The same chromatographic conditions were used to determine the enantiomeric excess (e.e.) for both enantiomers of all CDXs (**Table 22**). The e.e. values were determined by injecting the solutions of enantiomeric mixtures prepared mixing equal aliquots of each enantiomer and then each enantiomer separately. Solutions of each enantiomer contaminated with 1% of the opposite enantiomer were also prepared.

Table 22: Enantiomeric excess (e.e) of CDXs.

CDXs	e.e	CDXs	e.e
(*S*)-XEA-3-MET (**137**)	>99.9%	**(*S*)-XEA5-4-CLO** (**149**)	99.6%
(*R*)-XEA-3-MET (**138**)	99.8%	**(*R*)-XEA5-4-CLO** (**150**)	99.5%
(*S*)-XEA-4-MET (**139**)	99.9%	**(*S*)-XEA5-4-FLU** (**151**)	99.8%
(*R*)-XEA-4-MET (**140**)	99.9%	**(*R*)-XEA5-4-FLU** (**152**)	>99.9%
(*S*)-XEA-4-CLO (**141**)	>99.9%	**(*S*)-XEA5** (**153**)	99.2%
(*R*)-XEA-4-CLO (**142**)	99.5%	**(*R*)-XEA5** (**154**)	99.3%
(*S*)-XEA-4-FLU (**143**)	99.7%	**(*S*)-XEA5-DES** (**155**)	>99.9%
(*R*)-XEA-4-FLU (**144**)	99.2%	**(*R*)-XEA5-DES** (**156**)	>99.9%
(*S*)-XEA5-3-MET (**145**)	99.6%	**(*S,S*)-X2ADF5** (**157**)	99.1%
(*R*)-XEA5-3-MET (**146**)	99.3%	**(*R,R*)-X2ADF5** (**158**)	99.2%
(*S*)-XEA5-4-MET (**147**)	>99.9%	**(*S,R*)-X2ADF5** (**159**)	99.2%
(*R*)-XEA5-4-MET (**148**)	99.4%	**(*R,S*)-X2ADF5** (**160**)	99.2%

Mobile phase conditions: MeOH:ACN (50:50 *v/v*), flow rate 1.0 mL/min,.e.e = enantiomeric excess.

As shown in **Table 22** the e.e. values were higher than 99%. These results emphasize that do not occur racemization in the course of the synthesis. **Figure 23 - Figure 27** show an example of characteristic chromatograms obtained during the determination of the elution order and measuring of the e.e. **XEA-4-FLU** enantiomers**.**

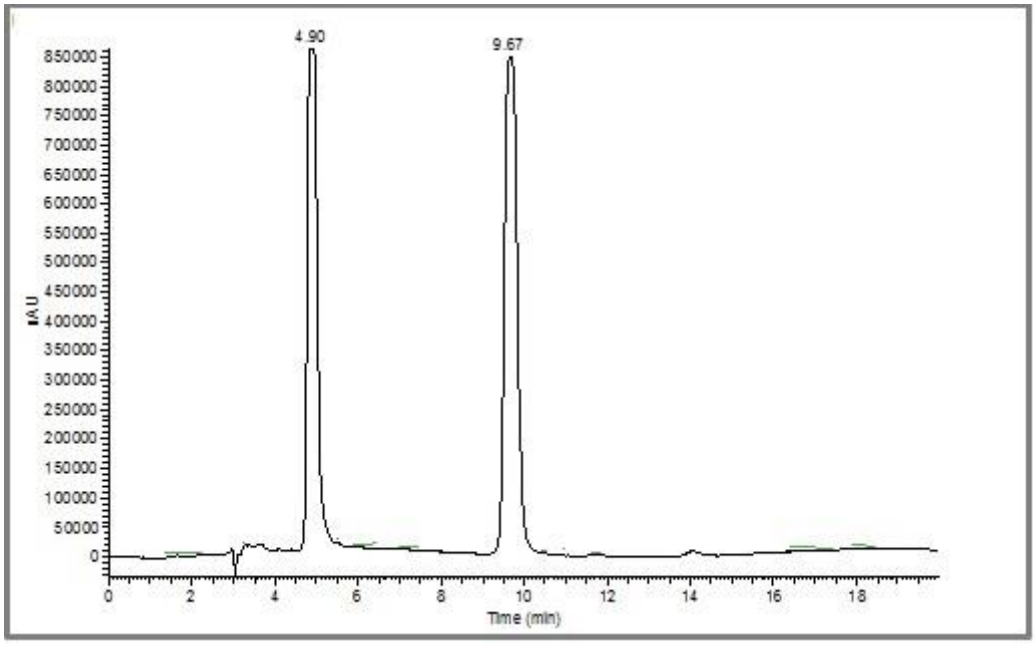


Figure 23: Chromatogram of enantiomeric mixture of **XEA-4-FLU**. Chromatographic conditions: column, (*S*,*S*)-Whelk-O1 CSP; mobile phase: MeOH:ACN (50:50 *v/v*); flow rate, 1.0 mL/min detection, 254 nm.

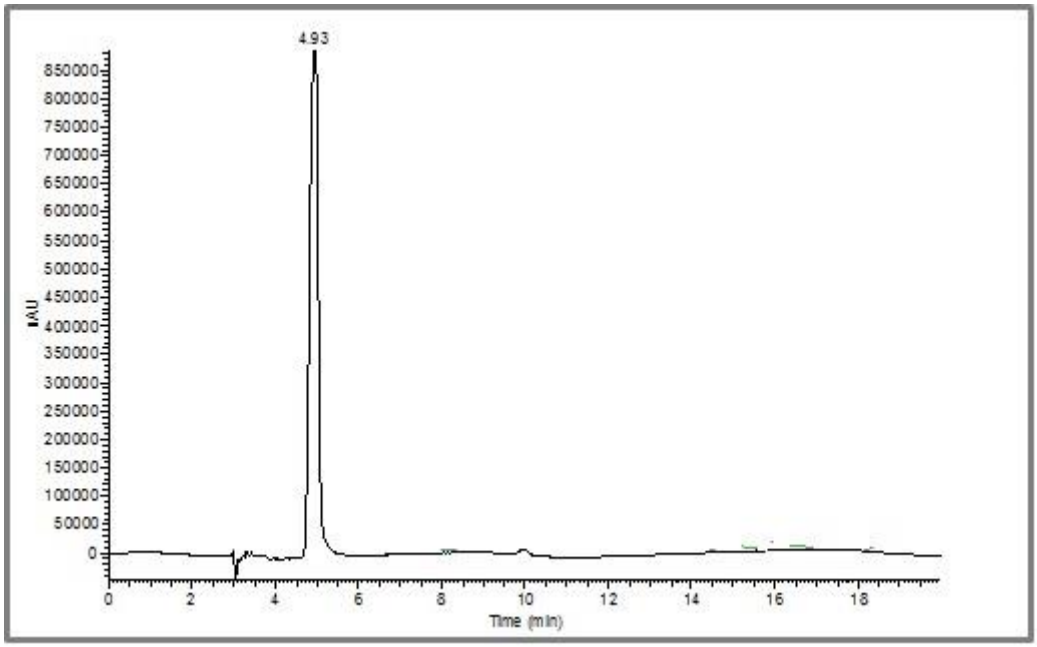


Figure 24: Chromatogram of (*R*)-enantiomer of **XEA-4-FLU** (**144**). Chromatographic conditions: column, (*S*,*S*)-Whelk-O1 CSP; mobile phase: MeOH:ACN (50:50 *v/v*); flow rate, 1.0 mL/min detection, 254 nm.

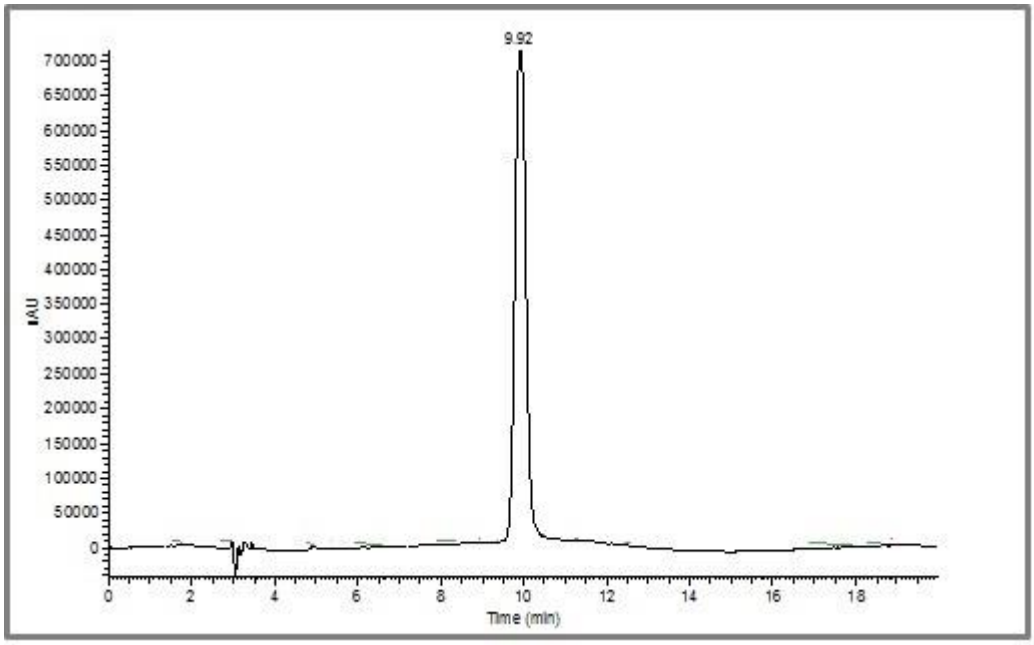


Figure 25: Chromatogram of (*S*)-enantiomer of **XEA-4-FLU** (**143**). Chromatographic conditions: column, (*S*,*S*)-Whelk-O1 CSP; mobile phase: MeOH:ACN (50:50 *v/v*); flow rate, 1.0 mL/min detection, 254 nm.

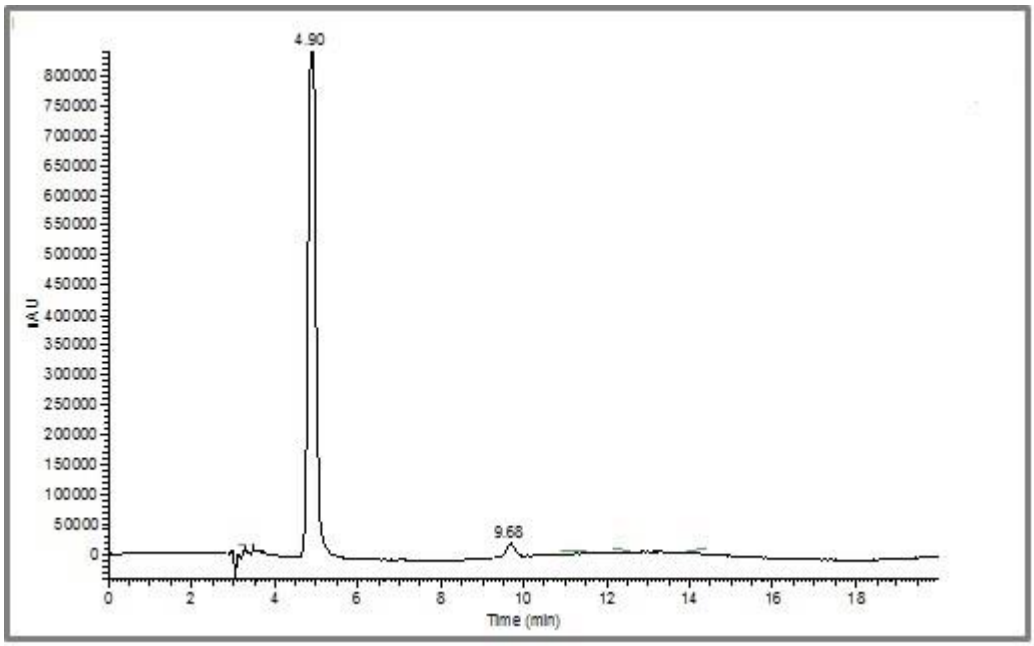


Figure 26: Chromatogram of (*R*)-enantiomer of **XEA-4-FLU** (**144**) spiked with 1% of (*S*)-enantiomer. Chromatographic conditions: column, (*S*,*S*)-Whelk-O1 CSP; mobile phase: MeOH:ACN (50:50 *v/v*); flow rate, 1.0 mL/min detection, 254 nm.

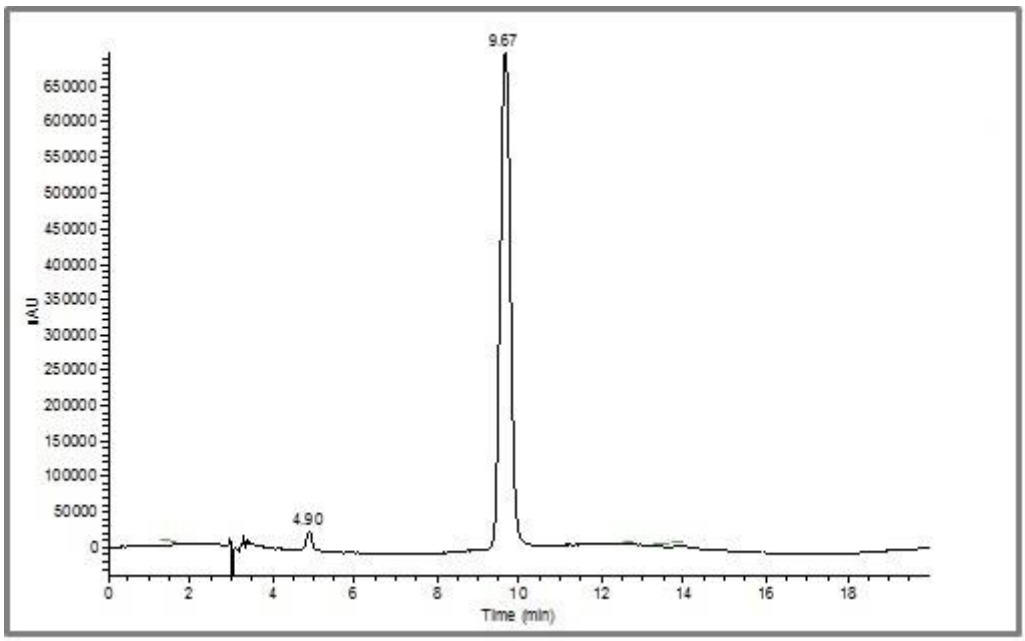


Figure 27: Chromatogram of (*S*)-enantiomer of **XEA-4-FLU** (**143**) spiked with 1% of (*R*)-enantiomer. Chromatographic conditions: column, (*S*,*S*)-Whelk-O1 CSP; mobile phase: MeOH:ACN (50:50 *v/v*); flow rate, 1.0 mL/min detection, 254 nm.

3. Biological activity

3.1. Effect on the growth of human tumor cell lines

The library of twenty-four new synthesized CDXs, in enantiomerically pure form, and the two xanthonic chemical substrates (Xcar-2, **108** and XCar-5, **110**) were tested in order to evaluated their effect on the *in vitro* growth of three human tumor cell lines: A375-C5 (melanoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer). The biological activity of the compounds was evaluated by Professor Hassan Bousbaa and co-workers. in Instituto Superior de Ciências da Saúde – Norte (ISCS-N) – CESPU.

This drug-screening procedure, adopted from the National Cancer Institute (NCI, USA), uses the protein binding dye sulforhodamine B, SRB,,to assess cell growth.[110] For each compound, a dose–response curve was established. The results, expressed as the concentration that was able to cause 50% cell growth inhibition (GI_{50}), are summarized in **Table 23**.

Table 23: Growth inhibitory activity of synthezised CDXs and XCars on the growth of three human tumor cell lines.

Compounds	GI_{50} (μM)		
	A375-C5	MCF-7	NCI-H460
XCar-2 (**108**)	113.75 ± 23.55	134.32 ± 10.34	>150
(***S***)**-XEA-3-MET** (**137**)	54.84 ± 46.02	92.55 ± 14.54	68.63 ± 5.91
(***R***)**-XEA-3-MET** (**138**)	>150	>150	>150
(***S***)**-XEA-4-MET** (**139**)	40.46 ± 6.02	37.35 ± 4.56	37.02 ± 4.75
(***R***)**-XEA-4-MET** (**140**)	59.99 ± 4.79	37.00 ± 7.67	38.80 ± 8.35
(***S***)**-XEA-4-CLO** (**141**)	72.72 ± 52.17	50.35 ± 40.02	86.55 ± 75.23
(***R***)**-XEA-4-CLO** (**142**)	89.31 ± 11.26	59.63 ± 4.91	87.24 ± 7.91
(***S***)**-XEA-4-FLU** (**143**)	>150	81.64 ± 13.43	104 ± 2.83
(***R***)**-XEA-4-FLU** (**144**)	137.5 ± 2.12	42.34 ± 31.61	70.33 ± 10.20
XCar-5 (**110**)	102.97 ± 22.24	90.51 ± 1.07	112.67 ± 12.34

Table 23: Continuation

(*S*)-XEA5-3-MET (**145**)	>150	>150	>150
(*R*)-XEA5-3-MET (**146**)	>150	>150	>150
(*S*)-XEA5-4-MET (**147**)	>150	99.69 ± 6.12	75.31 ± 5.36
(*R*)-XEA5-4-MET (**148**)	>150	85.89 ± 10.60	85.52 ± 16.47
(*S*)-XEA5-4-CLO (**149**)	63.44 ± 15.86	53.35 ± 0.43	48.41 ± 1.60
(*R*)-XEA5-4-CLO (**150**)	86.47 ± 6.04	69.35 ± 13.98	49.60 ± 9.74
(*S*)-XEA5-4-FLU (**151**)	>150	>150	>150
(*R*)-XEA5-4-FLU (**152**)	>150	133.50 ± 10.10	>150
(*S*)-XEA5 (**153**)	>150	93.59 ± 2.26	83.05 ± 13.16
(*R*)-XEA5 (**154**)	>150	>150	122.68 ± 21.00
(*S*)-XEA5-DES (**155**)	>150	>150	>150
(*R*)-XEA5-DES (**156**)	>150	>150	>150
(*S,S*)-X2ADF5 (**157**)	109.01 ± 8.20	75.28 ± 17.86	75.31 ± 18.13
(*R,R*)-X2ADF5 (**158**)	>150	84.35 ± 12.04	61.40 ± 21.32
(*S,R*)-X2ADF5 (**159**)	72.17 ± 7.60	38.75 ± 4.54	36.65 ± 7.58
(*R,S*)-X2ADF5 (**160**)	111.11 ± 21.52	50.38 ± 7.24	42.36 ± 5.63

*Results are expressed as mean ± SD of data obtained from three independent experiments.

The overall results obtained demonstrate that some CDXs exhibited interesting growth inhibitory effects on the tested human tumor cell lines. Actually, the most active CDX in all human tumor cell lines was compound **(*S*)-XEA-4-MET** (**139**) presenting values of GI_{50} = 37.02 ± 4.75 μM, GI_{50} = 37.35 ± 4.56 μM and GI_{50} = 40.46 ± 6.02 μM for NCI-H460, MCF-7 and A375-C5, respectively. Moreover, its antipode **(*R*)-XEA-4-MET** (**140**)

also revealed interesting inhibitory effects with values of GI_{50} = 38.80 ± 8.35 μM, GI_{50} = 37.00 ± 7.67 μM and GI_{50} = 59.99 ± 4.79 μM for NCI-H460, MCF-7 and A375-C5, respectively.

Others CDXs synthesized by coupling the xanthonic chemical substrate XCar-2 (**108**) with chiral amines as building blocks were also able to inhibit the growth of cell lines, such as **(*R*)-XEA-4-FLU** (**144**) with GI_{50} value of 42.34 ± 31.61 μM for MCF-7.

Among the synthesized CDXs obtained from XCar-5 (**110**), compound **(*S,R*)-X2ADF5** (**159**) presented the higher tumor cell growth inhibitory effect with values of GI_{50} = 36.65 ± 7.58 μM, and GI_{50} = 38.75 ± 4.54 μM in the cell lines NCI-H460 and MCF-7, respectively. Both enantiomers of **XEA5-4-MET** also revealed interesting results. For example, for the cell lines NCI-H460 and MCF-7 the (*S*)-enantiomer (**149**) showed values of GI_{50} = 75.31 ± 5.36 μM, and GI_{50} = 99.69 ± 6.12 μM, respectively; the values of GI_{50} for (*R*)-enantiomer (**150**) in the same cell lines were 85.52 ± 16.47 μM, and 85.89 ± 10.6 μM, respectively.

XCar-5 (**110**) possessing two methyl groups showed better results (although presenting a weaker activity) in all human tumor cell lines tested than XCar-2 (**108**) possessing one methoxy group. However, in general, the CDXs synthesized from XCar-2 (**108**) showed better results than the CDXs obtained from XCar-5 (**110**).

Furthermore, the evaluation of the growth inhibitory effect of the series of CDXs synthesized by coupling the carboxyxanthones XCar-2 (**108**) or XCar-5 (**110**) with different chiral amines allowed taking some considerations regarding structure–activity relationship.

For example, comparing the results obtained with the CDXs **(*S*)-XEA-4-MET** (**139**) and its enantiomer **(*R*)** (**140**) and **(*S*)-XEA5-4-MET** (**147**) and its enantiomer **(*R*)** (**148**), possessing the same chiral moiety while the xanthonic chemical substrates is the XCar-2 (**108**) and XCar-5 (**110**), respectively, it can be seen that: the first pair of compounds exhibited the highest growth inhibitory effects on the three human tumor cell lines, while the second pair was practically inactive. Therefore, it is interesting to emphasise that the nature of the substituents and their positions on the xanthonic chemical substrate also have an important role in growth inhibitory effect and enantioselectivity.

Concerning the influence of the configuration on the activity, several examples of enantioselectivity occurred between the enantiomeric pairs of CDXs obtained from XCar-2 on human tumor cell lines tested. For example, the CDX **(*R*)-XEA-3-MET** (**138**) was inactive for all cell lines tested (GI_{50} > 150 μM), while its enantiomer, **(*S*)-XEA-3-MET** (**137**) was more active presenting values of GI_{50} = 92.55 ± 14.54 μM, GI_{50} = 68.63 ± 5.91μM and GI_{50} = 54.84 ± 46.02 μM for MCF-7, NCI-H460 and A375-C5, respectively.

Another interesting example of enantioselectivity was observed between the enantiomers **(*S*)-XEA-4-FLU** (**143**) and **(*R*)-XEA-4-FLU** (**144**) in MCF-7 human tumor cell line. In fact, **(*S*)-XEA-4-FLU** (**143**) presented a slight inhibitory activity (GI_{50} = 81.64 ± 13.43 μM), while **(*R*)-XEA-4-FLU** (**144**) was able to inhibit the growth of this cell line with a GI_{50} = 42.34 ± 31.61μM. Considering these results as well as the incapacity of both enantiomers to inhibit the growth of the human tumor cell line A375-C5, it can be emphasized that cell-type selectivity also occurred.

Chapter 3:

Experimental

1. Chemistry

1.1. General Methods

All the synthesis were performed in Laboratory of Organic and Medicinal Chemistry of the Faculty of Pharmacy of the University of Porto.

The commercial available reagents and solvents were purchased from Sigma Aldrich Co and were used without purification.

All the reactions were monitored by thin-layer chromatography (Merck silica gel, 60 (GF_{254} plates), with appropriate mobile phases, and UV detection at 245 and 365 nm.

Purifications of compounds were carried out by flash chromatography using Macherey-Nagel silica gel 60 (0.04-0.063 mm), liquid-liquid extraction and crystallization.

Solvents were evaporated on a rotary evaporator under reduced pressure (rotative evaporator Büchi).

Optical rotation measurements were carried out on a Polartronic Universal polarimeter (ADP 410 polarimeter).

Melting points were obtained in a Köfler microscope and are uncorrect.

IR spectra were obtained in KBr microplate in a FTIR spectrometer Nicolet iS10 from Thermo Scientific (Waltham, MA, USA) with Smart OMNI-Transmisson accessory (Software 188 OMNIC 8.3).

^{1}H and ^{13}C NMR spectra were performed in the Department of Chemistry of the University of Aveiro and were taken using $CDCl_3$ or DMSO as solvent at room temperature, on Bruker Avance 300 and 500 instruments (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) used as an internal reference; ^{13}C NMR assignments were made by 2D (HSQC and HMBC) NMR experiments (long-range ^{13}C-^{1}H coupling constants were optimized to 7 Hz).

MS spectra were recorded as electronic impact mode on a VG Autospec Q spectrometer (*m/z*) and HRMS mass spectra were measured on a Bruker Daltonics micrOTOF Mass Spectrometer, recorded as electrospray modein Centro de Apoio Científico e Tecnológico á Investigation (C.A.C.T.I.), University of Vigo, Spain.

1.2. Synthesis of 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (XCar-2, 108) and 8-methoxy-9-oxo- 9*H*-xanthene-2-carboxylic acid (XCar-3, 109)

1.2.1. Esterification of 4-bromoisophthalic acid (111). Dimethyl 4-bromoisophthalate (112)

To a solution of 4-bromoisophthalic acid (**111**) (19.78 g, 79.62 mmol) in methanol (600 mL) was added 12 mL of concentrated H_2SO_4. Then, the reaction mixture was refluxed for 20 h. After evaporation of the methanol, water (65 mL) was added and the crude product was extracted with diethyl ether (3 x 70 mL). The organic layer was washed with water (100 mL), saturated $NaHCO_3$ solution (3 x 120 mL) and water (2 x 100 mL), successively. After drying with anhydrous sodium sulfate and filtered, the solvent was evaporated under reduced pressure. During overnight at room temperature the dimethyl 4-bromoisophthalate (**112**) appeared as a white solid. Yield: 92.3%; m.p. 56-58ºC; IR υ_{max} (cm^{-1}) (KBr): 1754, 1309, 1253, 929, 565; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 8.43 (1H, *d*, *J*= 2.2 Hz, H-2), 7.95 (1H, *dd*, *J*= 8.3 and 2.2 Hz, H-6), 7.75 (1H, *d*, *J*= 8.3 Hz, H-5), 3.95 (3H, *s*, C(1'')OOC$\underline{H}_3$), 3.93 (3H,*s*, C(1')OOC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 165.7 (C-1''), 165.5 (C-1'), 134.7 (C-4), 133.0 (C-6), 132.3 (C-2), 132.2 (C-5), 129.3 (C-3), 127.0 (C-1), 52.7 (C(1'')OO$\underline{C}$H$_3$), 52.5 (C(1')OO$\underline{C}$H$_3$); MS (EI) *m/z* (%): 273 $[M]^{+\cdot}$ (100), 256 (9), 240 (13), 221 (6), 209 (8), 203 (11).

1.2.2. Ullmann diaryl ether coupling. Dimethyl 4-(3-methoxyphenoxy) isophthalate (114)

A mixture of dimethyl 4-bromoisophthalate (**112**) (8.28 g, 30.33 mmol), 3-methoxyphenol (**113**) (4.5 mL, 36.39 mmol), CuI (0.29 g, 3.70 mmol), K_3PO_4 (12.86 g, 73.94 mmol) picolinic acid (1.14 g, 11.09 mmol) the sealed flask was then evacuated and backfilled with nitrogen. The evacuation/backfill sequence was repeated two additional times. Under nitrogen atmosphere remaining liquid reagents were added, followed by 3-methoxyphenol and dimethyl sulfoxide (61 mL).

The sealed flask was placed in a preheated oil bath at 80 °C and the reaction mixture was stirred vigorously with a magnetic stirrer for 28h. The reaction mixture was cooled to room temperature, filtered and extract with ethyl acetate (300 mL) and water (30 mL). The organic layer was washed with water (30 mL) and separated. The aqueous layer was extract twice more with ethyl acetate (20 mL). The combined organic layer were

washed with brine dried with anhydrous sodium sulfate, filtered, and evaporated under reduce pressure. The oily dark product was purified by flash chromatography (silica gel, petroleum ether/diethyl ether in gradient) to provide dimethyl 4-(3-methoxyphenoxy) isophthalate (**114**). Yield: 96%; m.p. 88-90 °C; IR υ_{max} (cm^{-1}) (KBr): 1724, 1719, 1613, 1488, 1274, 1229, 1153, 948, 763; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 8.58 (1H, *d*, *J*= 2.2 Hz, H-2), 8.07 (1H, *dd*, *J*=8.7 and 2.2 Hz, H-6), 7.27 (1H, *dd*, *J*= 8.4 and 8.3 Hz, H-5'), 6.95 (1H, *d*, *J*=8.7 Hz, H-5), 6.74 (1H, *dd*, *J*= 8.3 and 3.1 Hz, H-6'), 6.62 (1H, *dd*, *J*= 3.6 and 3.1 Hz, H-2'), 6.61 (1H, *dd*, *J*= 8.4 and 3.6 Hz, H-4'), 3.93 (3H, *s*, C(1')OOC$\underline{H}_3$), 3.89 (3H, *s*, C(1'')OOC$\underline{H}_3$), 3.79 (3H, *s*, Ar-OCH_3); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 165.7 (C-1'), 165.3 (C-1''), 161.1 (C-3), 160.4 (C-1), 156.9 (C-4), 134.6 (C-2), 133.6 (C-6), 130.4 (C-5), 124.6 (C-3'), 122.0 (C-1'), 118.7 (C-5'), 111.6 (C-6'), 110.3 (C-4'), 105.6 (C-2'), 55.4 (Ar-OCH_3), 52.3 (C(1')OO$\underline{C}H_3$), 52.2 (C(1')OO$\underline{C}H_3$); MS (EI) *m/z* (%): 316 $[M]^{+\cdot}$ (100), 285 $[M\text{-}OCH_3]^{+\cdot}$ (55), 253 (54), 242 (10), 225 (16), 213 (12), 198 (25), 138 (15), 127 (10), 92 (15), 83 (14), 64 (11).

1.2.3. Hydrolysis of dimethyl ester. 4-(3-Methoxyphenoxy)isophthalic acid (115)

Dimethyl 4-(3-methoxyphenoxy)isophthalate (**114**) (20.04 g, 63.35 mmol) was dissolved in methanol/tetrahydrofuran (1:1 *v/v*) and stirred at room temperature with 5M NaOH solution (67 mL) for 18 h. After evaporation of the organic solvents, water was added (400 mL) and the crude product was washed with ethyl acetate (2 x 200 mL). The organic layer was extracted with water (2 x 150 mL). The aqueous layer was acidified with 5M HCl solution resulting in the formation of a precipitate that was collected by filtration under reduced pressure and washed with cooled water, to provide 4-(3-methoxyphenoxy)isophthalic acid (**115**) as a white solid. Yield: 92% m.p. 232-234°C; IR υ_{max} (cm^{-1}) (KBr): 2907, 1694, 1680, 1601, 1486,1271,1265, 911, 758; ^{1}HNMR ($CDCl_3$, 300.13 MHz) δ: 8.53 (1H, *d*, *J*= 2.2 Hz, H-2), 8.09 (1H, *dd*, *J*= 8.7 and 2.2 Hz, H-6), 7.29 (1H, *dd*, *J*= 8.3 and 8.4 Hz, H-5'), 6.95 (1H, *d*, *J*= 8.7 Hz, H-5), 6.76 (1H, *dd*, *J*= 3.1 and 8.3 Hz, H-6'), 6.62 (1H, *dd*, *J*= 3.1 and 3.3 Hz, H-2'), 6.59 (1H, *dd*, *J*= 3.3 and 8.3 Hz, H-4'), 3.77 (3H, *s*, Ar-OC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 179.3 (C-1''), 179.3 (C-1'), 163.5 (C-3), 162.5 (C-1), 159.2 (C-4), 145.3 (C-2), 136.6 (C-6), 135.5 (C-5), 132.3 (C-3'), 127.3 (C-1'), 120.6 (C-5'), 113.4 (C-6'), 112.1 (C-4'), 107.5 (C-2'), 56.7(Ar-OCH_3); MS (EI) *m/z* (%): 288$[M]^{+\cdot}$ (100), 271 (10), 257$[M\text{-}OCH_3]^{+\cdot}$ (12), 227 (11), 199 (23), 165 (61), 124 (63), 92 (23), 77 (20), 64 (19).

1.2.4. Intramolecular acilation. Xanthones formation

To a solution of 4-(3'-methoxypenthoxy)isophthalic acid (**115**) (7.00 g, 14.40 mmol) in methane sulfonic acid (101.2 mL) was added phosphorus pentoxide (11.01 g, 45.99 mmol) and the reaction mixture was stirred at room temperature for 22 h. The mixture was poured over ice, resulting in the formation of a cream-coloured solid that was collected by filtration under reduced pressure and dried at room temperature. The crude product was dissolved in methanol (1200 mL) and H_2SO_4 (24 mL) was added. The mixture was refluxed for approximately 19h. The products methyl 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**116**) and methyl 8-methoxy-9-oxo-9*H*-xanthene-2-carboxylate (**117**) were separated by flash column chromatography (silica gel, petrolium ether:ethyl acetate in gradient). After that, compound **116** (2.08 g) was dissolved in methanol/dichloromethane (670 mL, 1:1 *v/v*) and 5M NaOH solution (29 mL) was added. The mixture was stirred at room temperature for 22 h. After evaporation of the organic solvents, water was added (100 mL) and the solution was acidified with 5M HCl solution resulting in the formation of a white precipitate. The suspension was filtered under reduced pressure and the white solid was washed with water, to afford 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid (**108**). Yield: 93%. The same procedure was followed to hydrolyse the methyl 8-methoxy-9-oxo-9H-xanthene-2-carboxylate (**109**) (37 mg, 0.14 mmol) to afford 8-methoxy-9-oxo-9H-xanthene-2-carboxylic acid (**108**). Yield: 1.5%

1.2.4.1. Methyl 6-methoxy-9-oxo-9H-xanthene-2-carboxylate (116) m.p.: 176-178 °C; IR υ_{max} (cm^{-1}) (KBr): 1730, 1663, 1581, 1467, 1438, 1270, 1117, 764; ^{1}HNMR ($CDCl_3$, 300.13 MHz) δ: 8.98 (1H, *d*, *J*= 2.1 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 8.24 (1H, *d*, *J*= 8.4 Hz, H-8), 7.48 (1H, *d*, *J*= 8.7 Hz, H-4), 6.96 (1H, *dd*, *J*= 2.3 and 8.4 Hz, H-7), 6.89 (1H, *d*, *J*= 2.3 Hz, H-5), 3.95 (3H, *s*, COOC$\underline{H}_3$), 3.93 (3H, *s*, Ar-OC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 175.5 (C-9), 165.9 ($\underline{C}OOCH_3$), 165.3 (C-6), 158.7 (C-10a), 157.8 (C-4a), 134.8 (C-3), 129.1 (C-2), 128.3 (C-8), 125.9 (C-1), 121.5 (C-9a), 118.1 (C-4), 115.6 (C-8a), 113.7 (C-7), 100.4 (C-5), 55.9 (Ar-O$\underline{C}H_3$), 52.3 (COO$\underline{C}H_3$); MS (EI) *m/z* (%): 284 $[M]^{+\cdot}$ (89), 253 $[M\text{-}OCH_3]^{+\cdot}$ (100), 225 (32), 197 (14), 182 (30), 169 (17), 154 (16), 142 (14), 126 (28), 111 (12), 75 (12), 63 (14).

1.2.4.2. Methyl 8-methoxy-9-oxo-9H-xanthene-2-carboxylate (117). m.p.: 206-207 °C; IR υ_{max} (cm^{-1}) (KBr): 1726, 1668, 1611, 1480, 1431, 1264, 1079, 760; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.98 (1H, *d*, *J*= 2.1 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 7.65 (1H, *dd*, *J*= 8.4 and 8.4 Hz, H-6), 7.47 (1H, *d*, *J*= 8.7 Hz, H-4), 7.09 (1H, *dd*, *J*= 0.8 and 8.4 Hz, H-7), 6.86 (1H, *dd*, *J*= 0.8 and 8.4 Hz, H-5), 4.05 (3H, *s*, COOC$\underline{H}_3$), 3.98 (3H, *s*, Ar-OC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.8 (C-9),166.0 ($\underline{C}OOCH_3$), 160.8 (C-8), 157.9 (C-10a), 157.8 (C-4a), 135.3 (C-3), 134.9 (C-6), 129.4 (C- 2), 126.0 (C-1), 122.6 (C-9a), 117.7 (C-4), 112.5 (C-8a), 110.1 (C-7), 106.1 (C-5), 56.5 (Ar-O$\underline{C}H_3$), 52.4 (COO$\underline{C}H_3$); MS (EI) *m/z* (%): 284 $[M]^{+\cdot}$ (100), 255 (56), 253 (30), 238 (56), 223 (47), 195 (27), 139 (33), 126 (20), 112 (18), 70 (15), 63 (12).

1.2.4.3. 6-Methoxy-9-oxo-9H-xanthene-2-carboxylic acid (108). m.p.: >300 °C; IR υ_{max} (cm^{-1}) (KBr): 3411, 1687, 1610, 1575, 1500, 1433,1271, 766; ^{1}H NMR (DMSO-d6, 300.13 MHz) δ: 8.68 (1H, *d*, *J*= 2.1 Hz, H-1), 8.29 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 8.09 (1H, *d*, *J*= 8.4 Hz, H-8), 7.69 (1H, *d*, *J*= 8.7 Hz, H-4), 7.19 (1H, *d*, *J*= 2.3 Hz, H-5), 7.07 (1H, *dd*, *J*= 2.3 and 8.4 Hz, H-7), 3.93 (3H, *s*, Ar-OC$\underline{H}_3$); ^{13}C NMR (DMSO-d6, 75.47 MHz) δ: 175.3 (C-9), 167.6 ($\underline{C}OOH$), 164.9 (C-6), 157.6 (C-10a), 156.3 (C-4a), 135.7 (C-3), 127.6 (C-2), 126.6 (C-8), 124.9 (C-1), 120.2 (C-9a), 116.7 (C-4), 115.0 (C-8a), 113.7 (C-7), 100.7 (C-5), 56.2 (Ar-O$\underline{C}H_3$); MS (EI) *m/z* (%): 270 $[M]^{+\cdot}$ (100), 253 $[M\text{-}OH]^{+\cdot}$ (26), 226 (27), 199 (15), 182 (15), 169 (8), 154 (7), 139 (7), 126 (17), 115 (10), 63 (16).

1.2.4.4. 8-Methoxy-9-oxo-9H-xanthene-2-carboxylic acid (109). m.p.: 268-270 °C; IR υ_{max} (cm^{-1}) (KBr): 3460, 1687, 1663, 1603, 1469, 1420, 1266, 763; ^{1}H NMR (DMSO-d6, 300.13MHz) δ: 8.66 (1H, *d*, *J*= 2.1 Hz, H-1), 8.28 (1H, *dd*, *J*= 2.1 and 8.7 Hz, H-3), 7.79 (1H, *dd*, *J*= 8.4 and 8.4 Hz, H-6), 7.67 (1H, *d*, *J*= 8.7 Hz, H-4), 7.20 (1H, *dd*, *J*= 0.7 and 8.4 Hz, H-7), 7.06 (1H, *dd*, *J*= 0.7 and 8.4 Hz, H-5), 3.94 (3H, *s*, Ar-OC$\underline{H}_3$); ^{13}C NMR (DMSO-d6, 125.77 MHz) δ: 174.3 (C-9), 166.2 ($\underline{C}OOH$), 160.2 (C-8), 157.2 (C-10a), 157.0 (C-4a), 136.1 (C-3), 134.9 (C-6), 127.9 (C-2), 125.0 (C-1), 122.1 (C-9a), 118.2 (C-4), 111.6 (C-8a), 109.7 (C-7), 107.0 (C-5), 56.3 (Ar-O$\underline{C}H_3$); HRMS (ESI) *m/z*: calcd for ($C_{15}H_{10}O_5$ + H): 271.16994, found: 271.06010.

1.3. Synthesis of 5,7-dimethyl-9-oxo-9H-xanthene-2-carboxylic acid (XCar-5, 110)

1.3.1. Esterification of 4-bromoisophthalic acid (111). Dimethyl 4-bromoisophthalate (112)

The dimethyl 4-bromoisophthalate (**112**) was synthesized (yield 90.7 %) and characterized according to the procedure described in **1.2.1**. (page 102).

1.3.2. Ullmann diaryl ether coupling. Dimethyl 4-(2,4-dimethylphenoxy) isophthalate (119)

A mixture of dimethyl 4-bromoisophthalate (**112**) (9.94 g, 36.39 mmol), 2,4-dimethylphenol (**118**) (8.0 mL, 54.59 mmol), CuI (0.35 g, 1.83 mmol), K_3PO_4 (15.53 g, 73.17 mmol) picolinic acid (0.45 g, 3.65 mmol) the sealed flask was then evacuated and backfilled with nitrogen. The evacuation/backfill sequence was repeated two additional times. Under nitrogen atmosphere remaining liquid reagents were added, followed by 2,4-dimethylphenol and dimethyl sulfoxide (73 mL). The sealed flask was placed in a preheated oil bath at 80 °C and the reaction mixture was stirred vigorously with a magnetic stirbar for 28h. The reaction mixture was cooled to room temperature, filtered and extracted with ethyl acetate (300 mL) and water (30 mL). The organic layer was washed with water (30 mL) and separated. The aqueous layer was extracting twice more with ethyl acetate (20 mL). The combined organic layer were washed with brine dried with anhydrous sodium sulfate, filtered, and evaporated under reduce pressure, the product dimethyl 4-(2,4-dimethylphenoxy) isophthalate (**119**) is oily dark. Yield: 87 %; m.p. 97-99 °C; IR υ_{max} (cm^{-1}) (KBr): 1717, 1436, 1259; ^{1}H NMR (DMSO-d_6, 300.13MHz) δ: 8.35 (1H, *d*, *J*= 2.2 Hz, H-2), 8.00 (1H, *dd*, *J*= 8.7 and 2.2 Hz, H-6), 7.17 (1H, *s*, H-3'), 7.08 (1H, *d*, *J*= 8.2 Hz, H-6'), 6.89 (1H, *d*, *J*= 8.2 Hz, H-5'), 6.70 (1H, *d*, *J*= 8.7 Hz, H-5), 3.84 (3H, *s*, C(3)OOC$\underline{H}_3$), 3.84 (3H, *s*, C(1)OOC$\underline{H}_3$), 2.29 (3H, *s*, C-4'-C$\underline{H}_3$), 2.07 (3H, *s*, C-2'-C$\underline{H}_3$); ^{13}C NMR (DMSO d6, 125.77 MHz) δ: 166.1 (C(3)OOCH$_3$), 166.0 (C(1)OOCH$_3$), 165.1 (C-3), 164.9 (C-1), 160.3 (C-4), 134.9 (C-6), 134.2 (C-2), 124.9 (C-1'), 124.4 (C-3'), 121.9 (C-5'), 120.3 (C-6'), 120.3 (C-5), 116.3 (C-2'), 116.2 (C-4'), 52.3 (C(3)OO$\underline{C}$H$_3$), 52.2 (C(1)OO$\underline{C}$H$_3$), 21.0 (C-2'-$\underline{C}$H$_3$), 20.4 (C-4'-$\underline{C}$H$_3$).

1.3.3. Hydrolysis of dimethyl ester. 4-(2,4-Dimethylphenoxy)isophthalic acid (120)

Dimethyl 4-(2,4-dimethylphenoxy) isophthalate (**119**) (21.37 g, 68.05 mmol) was dissolved in methanol/tetrahydrofuran (1:1 *v/v*) and stirred at room temperature with 5M NaOH solution (72 mL) for 18 h. After evaporation of the organic solvents, water was added (400 mL) and the crude product was washed with ethyl acetate (2 x 200 mL). The organic layer was extracted with water (2 x 150 mL). The aqueous layer was acidified with 5M HCl solution resulting in the formation of a precipitate that was collected by filtration under reduced pressure and washed with cooled water, to provide 4-(2,4-dimethylphenoxy)isophthalic acid **(120)** as a dark brown solid. Yield: 61 % m.p. 240-242 °C; IR υ_{max} (cm^{-1}) (KBr): 2923, 1613, 1604, 1491, 1257; ^{1}H NMR (DMSO-d_6, 300.13MHz) δ: 8.33 (1H, *d*, *J*= 2.2 Hz, H-2), 7.97 (1H, *dd*, *J*= 8.7 and 2.2 Hz, H-6), 7.16 (1H, *s*, H-3'), 7.07 (1H, *d*, *J*= 8.1 Hz, H-6'), 6.87 (1H, *d*, *J*= 8.1 Hz, H-5'), 6.67 (1H, *d*, *J*= 8.7 Hz, H-5), 2.29 (3H, *s*, C-4'-C$\underline{H}_3$), 2.07 (3H, *s*, C-2'-C$\underline{H}_3$); ^{13}C NMR (DMSO-d6, 125.77 MHz) δ: 166.3 (<u>C</u>(3)OOH), 166.2 (<u>C</u>(1)OOH), 165.1 (C-3), 164.2 (C-1), 160.0 (C-4), 134.4 (C-2), 132.9 (C-6), 128.2 (C-1'), 124.3 (C-3'), 122.0 (C-5'), 120.4 (C-6'), 120.3 (C-5), 118.1 (C-4'), 116.3 (C-2'), 20.4 (C-2'-<u>C</u>H_3), 15.5 (C-4'-<u>C</u>H_3).

1.3.4. Intramolecular acilation. Xanthone formation

To a solution of 4-(2,4-dimethylphenoxy)isophthalic acid **(120)** (7.87 g, 14.49 mmol) in methane sulfonic acid (114 mL) was added phosphorus pentoxide (12.36 g, 45.99 mmol) and the reaction mixture was stirred at room temperature for 22 h. The mixture was poured over ice, resulting in the formation of a cream-brown solid that was collected by filtration under reduced pressure and dried at room temperature to provide 5,7-dimethyl-9-oxo-9H-xanthene-2-carboxylic acid (XCar-5, **110**). Yield: 87%; m.p. >300 °C; IR υ_{max} (cm^{-1}) (KBr): 2920, 1667, 1611, 1475, 1421, 768, 680; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.61 (1H, *d*, *J*= 2.1 Hz, H-1), 8.23 (1H, *dd*, *J*= 8.7 and 2.1 Hz, H-3), 7.60 (1H, *d*, *J*= 8.7 Hz, H-4), 7.24 (1H, *s*, H-8), 7.02 (1H, *s*, H-6), 2.75 (3H, *s*, C-7-C$\underline{H}_3$), 2.50 (3H, *s*, C-5-C$\underline{H}_3$); ^{13}C NMR (DMSO-d6, 125.77 MHz) δ: 176.8 (C-9), 166.2 (C=OOH), 157.1 (C-10a), 156.8 (C-4a), 145.8 (C-7), 140.7 (C-5), 134.9 (C-3), 128.6 (C-6), 128.0 (C-1), 126.3 (C-2), 121.7 (C-9a), 118.2 (C-4), 117.1 (C-8a), 116.0 (C-8), 22.6 (C-5-C$\underline{H}_3$), 21.2 (C-7-C$\underline{H}_3$).

1.4. General procedure for the synthesis of chiral derivatives of xanthones

The 6-methoxy-9-oxo-9H-xanthene-2-carboxylic acid - XCar-2 (**108**) (100 mg, 0.37 mmol) or 5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid - XCar-5 (**110**) (100 mg, 0.37 mmol) was dissolved in dry THF (20 mL) and TEA (103 mL, 0.74 mmol) was added. Following, TBTU (120 mg, 0.37 mmol) and an appropriate chiral reagent (0.37 mmol) were added. The mixture was stirred at room temperature for 20 min or 1.5 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude product (**137-144**) was dissolved in ethyl acetate, (**145-156**) in dichloromethane, or (**157-160**) in chloroform (50 mL). This solution was washed with 1M HCl solution (2 X 25 mL), saturated solution of $NaHCO_3$ (2 X 30 mL) and water (3 X 50 mL). The organic layer was dried with anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure. The product was recrystallized from ethyl acetate/*n*-hexane (**137-144**) and from chloroform/*n*-hexane (**145-160**), to afford the CDX.

(*S*)-6-methoxy-N-(1-(3-methoxyphenyl)ethyl)-9-oxo-9H-xanthene-2-carboxamide - (*S*)-XEA-3-MET – (137) m.p.: 164-166°C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ -36.4 (c = 5.5 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1}) (KBr): 3319, 1663, 1631, 1533, 1482, 1469, 1440, 1270, 833; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.2 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.54 (1H, *d*, *J*= 8.8 Hz, H-4), 7.30 (1H, *d*, *J*= 7.9 Hz, H-2''), 7.00-6.98 (2H, *m*, H-5'' and H-6''), 6.97 (1H, dd, J= 2.3 and 8.9 Hz, H-7), 6.93 (1H, *d*, *J*= 2.3 Hz, H-5), 6.83 (1H, *ddd*, *J*= 0.7, 2.5 and 8.0 Hz, H-4''), 6.59 (1H, *d*, *J*= 7.8 Hz, N<u>H</u>), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OC<u>H</u>$_3$), 3.82 (3H, *s*, Ar-OC<u>H</u>$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.8 (C-6), 159.9 (C-3''), 158.0 (C-10a), 157.9 (C-4a), 144.5 (C-1''), 134.3 (C-3), 130.0 (C-2''), 129.8 (C-2), 128.4 (C-8), 124.0 (C-1), 121.1 (C-9a), 118.6 (C-6''), 118.5 (C-4), 115.5 (C-8a), 113.8 (C-7), 112.7 (C-4''), 112.3 (C-5''), 100.4 (C-5), 55.9 (Ar-O<u>C</u>H$_3$), 55.2 (Ar-O<u>C</u>H$_3$), 49.6 (C-1'), 21.8 (C-2').

(*R*)-6-methoxy-*N*-(1-(3-methoxyphenyl)ethyl)-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA-3-MET – (138) m.p.: 164-166°C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ +39.2 (c = 5.5 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1})(KBr): 3319, 1666, 1633, 1533, 1482, 1470, 1440, 1271, 833; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.2 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.54 (1H, *d*, *J*= 8.8 Hz, H-4), 7.30 (1H, *d*, *J*= 7.9 Hz, H-2''), 7.00-6.98 (2H, *m*, H-5'' and H-6''), 6.97 (1H, dd, J= 2.3 and 8.9 Hz, H-7), 6.93

(1H, *d*, *J*= 2.3 Hz, H-5), 6.83 (1H, *ddd*, *J*= 0.7, 2.5 and 8.0 Hz, H-4''), 6.58 (1H, *d*, *J*= 7.8 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OCH$_3$), 3.82 (3H, *s*, Ar-OCH$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.6 (C-6), 159.9 (C-3''), 158.0 (C-10a), 157.9 (C-4a), 144.5 (C-1''), 134.3 (C-3), 130.0 (C-2''), 129.9 (C-2), 128.4 (C-8), 124.0 (C-1), 121.1 (C-9a), 118.6 (C-6''), 118.6 (C-4), 115.5 (C-8a), 113.8 (C-7), 112.7 (C-4''), 112.3 (C-5''), 100.4 (C-5), 55.9 (Ar-OCH$_3$), 55.2 (Ar-OCH$_3$), 49.6 (C-1'), 21.8 (C-2').

(*S*)-6-methoxy-*N*-(1-(4-methoxyphenyl)ethyl)-9-oxo-9*H*-xanthene-2-carboxamide - (*S*)-XEA-4-MET – (139) m.p.: 210-212°C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ +26.5 (c = 5.6 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1}) (KBr): 3318, 1655, 1629, 1539, 1513, 1478, 1443, 1272, 831; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.56 (1H, *d*, *J*= 2.3 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.54 (1H, *d*, *J*= 8.8 Hz, H-4), 7.35 (2H, *d*, *J*= 8.5 Hz, H-2'' and H-6''), 6.99 (1H, dd, J= 2.4 and 8.9 Hz, H-7), 6.93 (1H, *d*, *J*= 2.7 Hz, H-5), 6.89 (2H, *d*, *J*= 2.1 Hz, H-3'' and H-5''), 6.59 (1H, *d*, *J*= 7.8 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OCH$_3$), 3.81 (3H, *s*, Ar-OCH$_3$), 1.63 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.8 (C-6), 159.0 (C-4''), 158.0 (C-10a), 157.9 (C-4a), 135.0 (C-1''), 134.3 (C-3), 130.0 (C-2), 128.3 (C-8), 127.5 (C-2'' and C-6''), 124.0 (C-1), 121.1 (C-9a), 118.5 (C-4), 115.5 (C-8a), 114.1(C-3'' and C-5''), 113.8 (C-7), 100.3 (C-5), 55.9 (Ar-OCH$_3$), 55.3 (Ar-OCH$_3$), 49.0 (C-1'), 21.6 (C-2').

(*R*)-6-methoxy-*N*-(1-(4-methoxyphenyl)ethyl)-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA-4-MET – (140) m.p.: 212-214°C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ -28.5 (c = 5.6 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1}) (KBr): 3319, 1658, 1636, 1537, 1479, 1443, 1272, 831; ; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.56 (1H, *d*, *J*= 2.3 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.54 (1H, *d*, *J*= 8.8 Hz, H-4), 7.35 (2H, *d*, *J*= 8.5 Hz, H-2'' and H-6''), 6.99 (1H, dd, J= 2.4 and 8.9 Hz, H-7), 6.93 (1H, *d*, *J*= 2.7 Hz, H-5), 6.89 (2H, *d*, *J*= 2.1 Hz, H-3'' and H-5''), 6.59 (1H, *d*, *J*= 7.8 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OCH$_3$), 3.81 (3H, *s*, Ar-OCH$_3$), 1.63 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.6 (C=O amide), 164.8 (C-6), 159.0 (C-4''), 158.0 (C-10a), 157.9 (C-4a), 135.0 (C-1''), 134.3 (C-3), 130.1 (C-2), 128.3 (C-8), 127.5 (C-2'' and C-6''), 124.0 (C-1), 121.0 (C-9a), 118.5 (C-4), 115.5 (C-8a), 114.1(C-3'' and C-5''), 113.8 (C-7), 100.3 (C-5), 55.9 (Ar-OCH$_3$), 55.3 (Ar-OCH$_3$), 49.0 (C-1'), 21.6 (C-2').

(*S*)-*N*-(1-(4-chlorophenyl)ethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide - (*S*)-XEA-4-CLO – (141) m.p.: 190-192 °C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ +136.7 (c = 5.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3290, 1651, 1635, 1589, 1540, 1480, 1445, 1275, 826; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.59 (1H, *d*, *J*= 2.2 Hz, H-1), 8.30 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.24 (1H, *d*, *J*= 8.9 Hz, H-8), 7.53 (1H, *d*, *J*= 8.8 Hz, H-4), 7.36-7.26 (4H, *m*, H-2'', H-3'', H-5'' and H-6''), 6.98 (1H, dd, J= 2.4 and 8.9 Hz, H-7), 6.91 (1H, *d*, *J*= 2.3 Hz, H-5), 6.71 (1H, *d*, *J*= 7.4 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.95 (3H, *s*, Ar-OCH_3), 1.62 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 165.0 (C-6), 158.0 (C-10a), 157.9 (C-4a), 141.6 (C-4''), 134.3 (C-3), 133.1 (C-1''), 129.8 (C-2), 128.3 (C-8), 127.6 (C-2'' and C-6''), 124.0 (C-1), 121.0 (C-9a), 118.6 (C-4), 117.8(C-3'' and C-5''), 115.5 (C-8a), 113.8 (C-7), 100.3 (C-5), 55.9 (Ar-O<u>C</u>H_3), 49.0 (C-1'), 21.8 (C-2').

(*R*)-*N*-(1-(4-chlorophenyl)ethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA-4-CLO – (142) m.p.: 190-192 °C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ -135.9 (c = 5.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1})(KBr): 3296, 1652, 1635, 1590, 1540, 1481, 1445, 1274, 827; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.59 (1H, *d*, *J*= 2.2 Hz, H-1), 8.30 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.24 (1H, *d*, *J*= 8.9 Hz, H-8), 7.53 (1H, *d*, *J*= 8.8 Hz, H-4), 7.36-7.26 (4H, *m*, H-2'', H-3'', H-5'' and H-6''), 6.98 (1H, dd, J= 2.4 and 8.9 Hz, H-7), 6.91 (1H, *d*, *J*= 2.3 Hz, H-5), 6.67 (1H, *d*, *J*= 7.4 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.95 (3H, *s*, Ar-OCH_3), 1.62 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.9 (C-6), 158.0 (C-10a), 157.9 (C-4a), 141.6 (C-4''), 134.3 (C-3), 133.1 (C-1''), 129.8 (C-2), 128.3 (C-8), 127.6 (C-2'' and C-6''), 124.0 (C-1), 121.0 (C-9a), 118.6 (C-4), 117.8(C-3'' and C-5''), 115.5 (C-8a), 113.8 (C-7), 100.3 (C-5), 55.9 (Ar-O<u>C</u>H_3), 49.0 (C-1'), 21.8 (C-2').

(*S*)-*N*-(1-(4-fluorophenyl)ethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide - (*S*)-XEA-4-FLU – (143) m.p.: 170-172 °C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ +45.5 (c = 5.7 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1}) (KBr): 3316, 1665, 1634, 1511, 1478, 1443, 1274, 833; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.57 (1H, *d*, *J*= 2.3 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.55 (1H, *d*, *J*= 8.8 Hz, H-4), 7.41–7.37 (2H, *m*, H-2'' and H-6''), 7.08-7.03 (2H, m, H-3''and H-5''), 6.99 (1H, dd, J= 2.3 and 8.9 Hz, H-7), 6.93 (1H, *d*, *J*= 2.3 Hz, H-5), 6.58 (1H, *d*, *J*= 7.4 Hz, NH), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OCH_3), 1.63 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.8 (C-6), 161.1 (C-4''), 158.0 (C-10a), 157.9 (C-4a), 138.7 (C-1''), 134.4 (C-3), 129.8 (C-2), 128.4 (C-8), 127.9 (C-2'' and C-6''), 123.9 (C-1), 121.0 (C-9a),

118.7 (C-4), 115.5 (C-3'' and C-5''), 115.6 (C-8a), 113.9 (C-7), 100.4 (C-5), 55.9 (Ar-O<u>C</u>H_3), 48.9 (C-1'), 21.8 (C-2').

(*R*)-*N*-(1-(4-fluorophenyl)ethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA-4-FLU – (144) m.p.: 170-172 °C (ethyl acetate/ *n*-hexane); $[\alpha]_D^{25°C}$ -52.5 (c = 5.7 x 10^{-3} g/mL in acetone); IR υ_{max} (cm^{-1}) (KBr): 3317, 1665, 1630, 1539, 1443, 1274, 834; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.57 (1H, *d*, *J*= 2.3 Hz, H-1), 8.32 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 8.26 (1H, *d*, *J*= 8.9 Hz, H-8), 7.55 (1H, *d*, *J*= 8.8 Hz, H-4), 7.41–7.37 (2H, *m*, H-2'' and H-6''), 7.08-7.03 (2H, m, H-3''and H-5''), 6.99 (1H, dd, J= 2.3 and 8.9 Hz, H-7), 6.93 (1H, *d*, *J*= 2.3 Hz, H-5), 6.58 (1H, *d*, *J*= 7.4 Hz, N<u>H</u>), 5.37-5.28 (1H, *m,* H-1'), 3.96 (3H, *s*, Ar-OC<u>H</u>$_3$), 1.63 (3H, *d*, *J*= 7.0 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 175.9 (C-9), 165.5 (C=O amide), 164.8 (C-6), 161.1 (C-4''), 158.0 (C-10a), 157.9 (C-4a), 138.7 (C-1''), 134.4 (C-3), 129.8 (C-2), 128.3 (C-8), 127.9 (C-2'' and C-6''), 123.9 (C-1), 121.0 (C-9a), 118.7 (C-4), 115.5 (C-3'' and C-5''), 115.6 (C-8a), 113.9 (C-7), 100.4 (C-5), 55.9 (Ar-O<u>C</u>H_3), 48.9 (C-1'), 21.8 (C-2').

(*S*)-*N*-(1-(3-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide-(*S*)-XEA5-3-MET – (145) m.p.: 195-197 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +183.7 (c = 3.7 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3289, 1664, 1633, 1606, 1540, 1473, 837, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.60 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.95 (1H, *d*, *J*= 0.8 Hz, H-8), 7.59 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.27 (1H, *d*, *J*= 7.9 Hz, H-2''), 7.02–6.96 (2H, *m*, H-5'' and H-6''), 6.82 (1H, *ddd*, *J*= 0.8, 2.6 and 8.0 Hz H-4''), 6.63 (1H, *d*, *J*= 7.6 Hz, N<u>H</u>), 5.37-5.27 (1H, *m,* H-1'), 3.81 (3H, *s*, Ar-OC<u>H</u>$_3$), 2.54 (3H, *s*, C5-C<u>H</u>$_3$), 2.44 (3H, *s*, C7-C<u>H</u>$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.9 (C=O amide), 159.8 (C-3''), 157.7 (C-4a), 152.7 (C-10a), 144.6 (C-1''), 137.6 (C-6), 134.5 (C-3), 133.8 (C-7), 129.8 (C-2), 127.1 (C-5), 124.2 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 118.5 (C-5''), 112.7 (C-6''), 112.3 (C-2''), 112.3 (C-4''), 55.2 (Ar-O<u>C</u>H_3), 49.5 (C-1'), 21.7 (C-2'), 20.8 (C7-<u>C</u>H_3), 15.7 (C5-<u>C</u>H_3).

(*R*)-*N*-(1-(3-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA5-3-MET – (146) m.p.: 195-197 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -173.5 (c = 3.7 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3297, 1664, 1633, 1606, 1540, 1473, 837, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.59 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.95 (1H, *d*, *J*= 0.8 Hz, H-8), 7.59 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.27 (1H, *d*, *J*= 7.9 Hz, H-2''), 7.02–6.96 (2H, *m*, H-5'' and H-6''), 6.82 (1H,

ddd, *J*= 0.8, 2.6 and 8.0 Hz H-4''), 6.63 (1H, *d*, *J*= 7.6 Hz, NH), 5.37-5.27 (1H, *m,* H-1'), 3.81 (3H, *s*, Ar-OCH$_3$), 2.54 (3H, *s*, C5-CH$_3$), 2.44 (3H, *s*, C7-CH$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.9 (C=O amide), 159.8 (C-3''), 157.7 (C-4a), 152.7 (C-10a), 144.6 (C-1''), 137.6 (C-6), 134.5 (C-3), 133.8 (C-7), 129.8 (C-2), 127.1 (C-5), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 118.5 (C-5''), 112.7 (C-6''), 112.3 (C-2''), 112.3 (C-4''), 55.2 (Ar-OCH$_3$), 49.5 (C-1'), 21.7 (C-2'), 20.8 (C7-CH$_3$), 15.7 (C5-CH$_3$).

(*S*)-*N*-(1-(4-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*S*)-XEA5-4-MET – (147) m.p.: 188-190 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +179.3 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3303, 1662, 1634, 1614, 1539, 1515, 1466, 829, 792; ; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.57 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.96 (1H, *d*, *J*= 0.8 Hz, H-8), 7.59 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.35 (2H, *d*, *J*= 8.7 Hz, H-2'' and H-6''), 6.90 (2H, *d*, *J*= 8.7 Hz H-3'' and H-5''), 6.60 (1H, *d*, *J*= 7.5 Hz, NH), 5.37-5.27 (1H, *m,* H-1'), 3.80 (3H, *s*, Ar-OCH$_3$), 2.54 (3H, *s*, C5-CH$_3$), 2.44 (3H, *s*, C7-CH$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.8 (C=O amide), 158.9 (C-4''), 157.7 (C-4a), 152.7 (C-10a), 137.6 (C-6), 135.0 (C-1''), 134.5 (C-3), 133.7 (C-7), 129.8 (C-2), 127.5 (C-2'' and C-6''), 127.1 (C-5), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 114.1 (C-3'' and C-5''), 55.3 (Ar-OCH$_3$), 49.0 (C-1'), 21.6 (C-2'), 20.8 (C7-CH$_3$), 15.7 (C5-CH$_3$).

(*R*)-*N*-(1-(4-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*R*)-XEA5-4-MET – (148) m.p.: 188-190 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -177.6 (c = 4.4 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3300, 1662, 1633, 1610, 1550, 1467, 829, 793; ; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.57 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.96 (1H, *d*, *J*= 0.8 Hz, H-8), 7.59 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.35 (2H, *d*, *J*= 8.7 Hz, H-2'' and H-6''), 6.90 (2H, *d*, *J*= 8.7 Hz H-3'' and H-5''), 6.60 (1H, *d*, *J*= 7.5 Hz, NH), 5.37-5.27 (1H, *m,* H-1'), 3.80 (3H, *s*, Ar-OCH$_3$), 2.54 (3H, *s*, C5-CH$_3$), 2.44 (3H, *s*, C7-CH$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.8 (C=O amide), 158.9 (C-4''), 157.7 (C-4a), 152.7 (C-10a), 137.6 (C-6), 135.0 (C-1''), 134.5 (C-3), 133.7 (C-7), 129.8 (C-2), 127.5 (C-2'' and C-6''), 127.1 (C-5), 124.0 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 114.1 (C-3'' and C-5''), 55.3 (Ar-OCH$_3$), 49.0 (C-1'), 21.6 (C-2'), 20.8 (C7-CH$_3$), 15.7 (C5-CH$_3$).

(*S*)-*N*-(1-(4-chlorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*S*)-XEA5-4-CLO – (149) m.p.: 215-217 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +204.2 (c = 4.7 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3297, 1667, 1629, 1611, 1536, 1474, 825, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.60 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.95 (1H, *d*, *J*= 0.7 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.36 (2H, *d*, *J*= 8.8 Hz, H-2'' and H-6''), 7.31 (2H, *d*, *J*= 8.8 Hz H-3'' and H-5''), 6.65 (1H, *d*, *J*= 7.5 Hz, NH), 5.37-5.27 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-CH_3), 2.44 (3H, *s*, C7-CH_3), 1.63 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 165.0 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 141.6 (C-4''), 137.7 (C-6), 134.5 (C-3), 133.8 (C-7), 133.2 (C-1''), 129.6 (C-2), 128.8 (C-2'' and C-6''), 127.7 (C-3'' and C-5''), 127.2 (C-5), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 119.0 (C-4), 49.0 (C-1'), 21.8 (C-2'), 20.8 (C7-CH_3), 15.7 (C5-CH_3).

(*R*)-*N*-(1-(4-chlorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*R*)-XEA5-4-CLO – (150) m.p.: 215-217 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -203.4 (c = 4.7 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3299, 1666, 1631, 1611, 1540, 1470, 828, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.60 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.95 (1H, *d*, *J*= 0.7 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.36 (2H, *d*, *J*= 8.8 Hz, H-2'' and H-6''), 7.31 (2H, *d*, *J*= 8.8 Hz H-3'' and H-5''), 6.65 (1H, *d*, *J*= 7.5 Hz, NH), 5.37-5.27 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-CH_3), 2.44 (3H, *s*, C7-CH_3), 1.63 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.9 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 141.6 (C-4''), 137.7 (C-6), 134.5 (C-3), 133.8 (C-7), 133.2 (C-1''), 129.5 (C-2), 128.8 (C-2'' and C-6''), 127.7 (C-3'' and C-5''), 127.2 (C-5), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 119.0 (C-4), 49.0 (C-1'), 21.8 (C-2'), 20.8 (C7-CH_3), 15.7 (C5-CH_3).

(*S*)-*N*-(1-(4-fluorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide - (*S*)-XEA5-4-FLU – (151) m.p.: 186-188 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +167.4 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3304, 1673, 1637, 1609, 1555, 1473, 824, 790; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.98 (1H, *d*, *J*= 0.7 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.41 (2H, *d*, *J*= 8.7 Hz, H-2'' and H-6''), 7.07 (2H, *d*, *J*= 8.7 Hz H-3'' and H-5''), 6.58 (1H, *d*, *J*= 7.5 Hz, NH), 5.38-5.28 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-CH_3), 2.44 (3H, *s*, C7-CH_3), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 165.0 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 138.8 (C-4''), 137.7 (C-6), 135.0 (C-1''), 134.5 (C-3), 133.9 (C-7), 129.7 (C-2), 128.0 (C-2'' and C-6''), 127.2 (C-5), 124.1 (C-1), 123.6 (C-8),

121.1 (C-8a), 120.6 (C-9a), 119.0 (C-4), 115.6 (C-3'' and C-5''), 48.9 (C-1'), 21.8 (C-2'), 20.8 (C7-$\underline{C}H_3$), 15.7 (C5-$\underline{C}H_3$).

(*R*)-*N*-(1-(4-fluorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide - (*R*)-XEA5-4-FLU – (152) m.p.: 186-188 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -166.7 (c = 4.2 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3303, 1672, 1637, 1608, 1555, 1473, 829, 792; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.98 (1H, *d*, *J*= 0.7 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.8 Hz, H-6), 7.41 (2H, *d*, *J*= 8.7 Hz, H-2'' and H-6''), 7.07 (2H, *d*, *J*= 8.7 Hz H-3'' and H-5''), 6.58 (1H, *d*, *J*= 7.5 Hz, N$\underline{H}$), 5.38-5.28 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-C$\underline{H}_3$), 2.44 (3H, *s*, C7-C$\underline{H}_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 165.0 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 138.8 (C-4''), 137.7 (C-6), 135.0 (C-1''), 134.5 (C-3), 133.9 (C-7), 129.7 (C-2), 128.0 (C-2'' and C-6''), 127.2 (C-5), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 119.0 (C-4), 115.6 (C-3'' and C-5''), 48.9 (C-1'), 21.8 (C-2'), 20.8 (C7-$\underline{C}H_3$), 15.7 (C5-$\underline{C}H_3$).

(*S*)-5,7-dimethyl-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide – (*S*)-XEA5 - (153) m.p.: 210-212 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +130.7 (c = 3.9 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3298, 1667, 1632, 1609, 1547, 1466, 834, 790; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.9 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.9 Hz, H-6), 7.33 (2H, *d*, *J*= 8.0 Hz, H-2'' and H-6''), 7.18 (2H, *d*, *J*= 8.0 Hz H-3'' and H-5''), 6.60 (1H, *d*, *J*= 7.6 Hz, N$\underline{H}$), 5.37-5.27 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-C$\underline{H}_3$), 2.44 (3H, *s*, C7-C$\underline{H}_3$), 2.34 (3H, *s*, amine-C$\underline{H}_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.8 (C=O amide), 157.7 (C-4a), 152.7 (C-10a), 139.9 (C-4''), 137.6 (C-6), 137.2 (C-1''), 134.5 (C-3), 133.8 (C-7), 129.9 (C-2), 129.4 (C-2'' and C-6''), 127.1 (C-5), 126.2 (C-3'' and C-5''), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 49.4 (C-1'), 21.7 (amine-$\underline{C}H_3$), 21.1 (C-2'), 20.8 (C7-$\underline{C}H_3$), 15.7 (C5-$\underline{C}H_3$).

(*R*)-5,7-dimethyl-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide – (*R*)-XEA5 – (154) m.p.: 210-212 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -125.8 (c = 3.9 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3300, 1667, 1634, 1612, 1542, 1466, 832, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.58 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.9 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43 (1H, *d*, *J*= 0.9 Hz, H-6), 7.33 (2H, *d*, *J*= 8.0 Hz, H-2'' and H-6''), 7.18 (2H, *d*, *J*= 8.0 Hz H-3'' and H-5''), 6.60 (1H, *d*, *J*= 7.6 Hz, N$\underline{H}$), 5.37-5.27 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-C$\underline{H}_3$), 2.44 (3H, *s*, C7-C$\underline{H}_3$), 2.34 (3H,

s, amine-C<u>H</u>$_3$), 1.64 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.8 (C=O amide), 157.9 (C-4a), 152.7 (C-10a), 139.9 (C-4''), 137.6 (C-6), 137.2 (C-1''), 134.5 (C-3), 133.8 (C-7), 129.9 (C-2), 129.4 (C-2'' and C-6''), 127.1 (C-5), 126.2 (C-3'' and C-5''), 124.4 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 49.4 (C-1'), 21.7 (amine-<u>C</u>H$_3$), 21.1 (C-2'), 20.8 (C7-<u>C</u>H$_3$), 15.7 (C5-<u>C</u>H$_3$).

(*S*)-5,7-dimethyl-9-oxo-*N*-(1-phenylethyl)-9*H*-xanthene-2-carboxamide – (*S*)-XEA5-DES – (155) m.p.: 199-201 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +132.5 (c = 4.0 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3303, 1666, 1630, 1611, 1536, 1475, 833, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.59 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.9 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43-7.27 (6H, *d*, *J*= 8.0 Hz, H-6, H-2'', H-3'', H-4'', H-5'' and H-6''), 6.65 (1H, *d*, *J*= 7.6 Hz, N<u>H</u>), 5.41-5.32 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-C<u>H</u>$_3$), 2.44 (3H, *s*, C7-C<u>H</u>$_3$), 1.66 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.9 (C=O amide), 157.7 (C-4a), 152.7 (C-10a), 142.9 (C-1''), 137.6 (C-6), 134.5 (C-3), 133.8 (C-7), 129.8 (C-2), 128.7 (C-3'' and C-5''), 127.5 (C-4''),127.1 (C-5), 126.3 (C-2'' and C-6''), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 49.5 (C-1'), 21.7 (C-2'), 20.8 (C7-<u>C</u>H$_3$), 15.7 (C5-<u>C</u>H$_3$).

(*R*)-5,7-dimethyl-9-oxo-*N*-(1-phenylethyl)-9*H*-xanthene-2-carboxamide – (*R*)-XEA5-DES – (156) m.p.: 199-201 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -131.5 (c = 4.0 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3304, 1665, 1632, 1612, 1536, 1475, 837, 791; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 8.59 (1H, *d*, *J*= 2.3 Hz, H-1), 8.33 (1H, *dd*, *J*= 2.3 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.9 Hz, H-8), 7.60 (1H, *d*, *J*= 8.8 Hz, H-4), 7.43-7.27 (6H, *d*, *J*= 8.0 Hz, H-6, H-2'', H-3'', H-4'', H-5'' and H-6''), 6.65 (1H, *d*, *J*= 7.6 Hz, N<u>H</u>), 5.41-5.32 (1H, *m,* H-1'), 2.54 (3H, *s*, C5-C<u>H</u>$_3$), 2.44 (3H, *s*, C7-C<u>H</u>$_3$), 1.66 (3H, *d*, *J*= 6.9 Hz, H-2'). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.3 (C-9), 164.9 (C=O amide), 157.7 (C-4a), 152.7 (C-10a), 142.9 (C-1''), 137.6 (C-6), 134.5 (C-3), 133.8 (C-7), 129.8 (C-2), 128.7 (C-3'' and C-5''), 127.5 (C-4''),127.1 (C-5), 126.3 (C-2'' and C-6''), 124.1 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 49.5 (C-1'), 21.7 (C-2'), 20.8 (C7-<u>C</u>H$_3$), 15.7 (C5-<u>C</u>H$_3$).

***N*-((1*S*,2*S*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*S*,*S*)-X2ADF5 – (157)** m.p.: 243-245 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -30.2 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3421, 3320, 1669, 1634, 1613, 1529, 1475, 792, 701; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 9.01 (1H, *d*, *J*= 8.3 Hz, N<u>H</u>), 8.55 (1H, *d*, *J*= 2.1 Hz, H-1), 8.09 (1H, *dd*, *J*= 2.1 and 8.8 Hz, H-3), 7.82 (1H, *d*, *J*= 0.8 Hz, H-8), 7.68 (1H, *d*, *J*= 8.8 Hz, H-4), 7.59 (1H, *d*, *J*= 0.8 Hz, H-6), 7.33-7.16 (10H, *m*, H-2'',

H-3'', H-4'', H-5'', H-6'', H-2''', H-3''', H-4''', H-5''' and H-6'''), 5.70 (1H, *d*, *J*= 5.1 Hz, OH), 5.21-5.15 (1H, *m,* H-1'), 4.99 (3H, *d*, *J*= 5.1 Hz, H-2'), 2.51 (3H, *s*, C5-CH_3), 2.48 (3H, *s*, C7-CH_3). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 176.1 (C-9), 163.8 (C=O amide), 156.9 (C-4a), 152.7 (C-10a), 143.6 (C-1''), 141.3 (C-1'''), 137.6 (C-6), 134.0 (C-3), 133.5 (C-7), 130.2 (C-2), 128.4 (C-3'' and C-5''), 127.7 (C-2'' and C-6''), 127.7 (C-3''' and C-5'''), 127.0 (C-2''' and C-6'''), 127.0 (C-5), 126.9 (C-4''), 126.6 (C-4'''), 125.5 (C-1), 122.9 (C-8), 120.6 (C-8a), 120.1 (C-9a), 118.5 (C-4), 74.5 (C-2'), 59.2 (C-1'), 20.3 (C7-CH_3), 15.2 (C5-CH_3).

***N*-((1*R*,2*R*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*R*,*R*)-X2ADF5 – (158)** m.p.: 243-245 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +52.8 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3389, 3302, 1668, 1635, 1533, 1489, 1475, 801, 702; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 9.18 (1H, *d*, *J*= 8.3 Hz, NH), 8.76 (1H, *d*, *J*= 2.1 Hz, H-1), 7.86 (1H, *d*, *J*= 0.8 Hz, H-8), 7.76 (1H, *dd*, *J*= 2.1 and 8.8 Hz, H-3), 7.76 (1H, *d*, *J*= 8.8 Hz, H-4), 7.61 (1H, *d*, *J*= 0.8 Hz, H-6), 7.32-7.14 (10H, *m*, H-2'', H-3'', H-4'', H-5'', H-6'', H-2''', H-3''', H-4''', H-5''' and H-6'''), 5.70 (1H, *d*, *J*= 5.1 Hz, OH), 5.26-5.21 (1H, *m,* H-1'), 4.99 (3H, *d*, *J*= 5.1 Hz, H-2'), 2.51 (3H, *s*, C5-CH_3), 2.48 (3H, *s*, C7-CH_3). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 176.2 (C-9), 164.6 (C=O amide), 156.9 (C-4a), 152.7 (C-10a), 143.0 (C-1''), 141.3 (C-1'''), 137.6 (C-6), 134.2 (C-3), 133.5 (C-7), 130.2 (C-2), 127.8 (C-3'' and C-5''), 127.7 (C-2'' and C-6''), 127.7 (C-3''' and C-5'''), 127.0 (C-2''' and C-6'''), 127.0 (C-5), 126.9 (C-4''), 126.6 (C-4'''), 125.5 (C-1), 122.9 (C-8), 120.7 (C-8a), 120.1 (C-9a), 118.5 (C-4), 75.5 (C-2'), 59.2 (C-1'), 20.3 (C7-CH_3), 15.2 (C5-CH_3).

***N*-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*S*,*R*)-X2ADF5 – (159)** m.p.: 240-242 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ +32.2 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3385, 3311, 1673, 1635, 1533, 1474, 755, 701; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 9.18 (1H, *d*, *J*= 8.3 Hz, NH), 8.64 (1H, *d*, *J*= 2.1 Hz, H-1), 8.20 (1H, *dd*, *J*= 2.1 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.8 Hz, H-8), 7.56 (1H, *d*, *J*= 8.8 Hz, H-4), 7.44 (1H, *d*, *J*= 0.8 Hz, H-6), 7.38-7.24 (10H, *m*, H-2'', H-3'', H-4'', H-5'', H-6'', H-2''', H-3''', H-4''', H-5''' and H-6'''), 5.70 (1H, *d*, *J*= 5.1 Hz, OH), 5.46-5.42 (1H, *m,* H-1'), 5.16 (3H, *d*, *J*= 5.1 Hz, H-2'), 2.53 (3H, *s*, C5-CH_3), 2.45 (3H, *s*, C7-CH_3). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.2 (C-9), 166.0 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 140.7 (C-1''), 139.4 (C-1'''), 137.6 (C-6), 134.4 (C-3), 133.8 (C-7), 129.6 (C-2), 127.9 (C-2'' and C-6''), 127.8 (C-3'' and C-5''), 127.8 (C-2''' and C-6'''), 127.4 (C-3''' and C-5'''), 127.1 (C-5), 127.0 (C-4''), 126.1 (C-4'''), 125.5 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 74.9 (C-2'), 60.2 (C-1'), 20.8 (C7-CH_3), 15.7 (C5-CH_3).

***N*-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide – (*R*,*S*)-X2ADF5 – (160)** m.p.: 241-243 °C (chloroform/ *n*-hexane); $[\alpha]_D^{25°C}$ -39.0 (c = 4.3 x 10^{-3} g/mL in chloroform); IR υ_{max} (cm^{-1}) (KBr): 3418, 3311, 1674, 1636, 1614, 1540, 1474, 754, 700; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 9.18 (1H, *d*, *J*= 8.3 Hz, NH), 8.64 (1H, *d*, *J*= 2.1 Hz, H-1), 8.29 (1H, *dd*, *J*= 2.1 and 8.8 Hz, H-3), 7.97 (1H, *d*, *J*= 0.8 Hz, H-8), 7.59 (1H, *d*, *J*= 8.8 Hz, H-4), 7.44 (1H, *d*, *J*= 0.8 Hz, H-6), 7.38-7.24 (10H, *m*, H-2'', H-3'', H-4'', H-5'', H-6'', H-2''', H-3''', H-4''', H-5''' and H-6'''), 5.70 (1H, *d*, *J*= 5.1 Hz, OH), 5.53-5.49 (1H, *m,* H-1'), 5.16 (3H, *d*, *J*= 5.1 Hz, H-2'), 2.53 (3H, *s*, C5-CH_3), 2.45 (3H, *s*, C7-CH_3). ^{13}C NMR ($CDCl_3$,125.77 MHz) δ: 177.2 (C-9), 165.6 (C=O amide), 157.8 (C-4a), 152.7 (C-10a), 140.7 (C-1''), 139.7 (C-1'''), 137.6 (C-6), 134.4 (C-3), 133.8 (C-7), 129.6 (C-2), 128.2 (C-3'' and C-5''), 128.2 (C-3''' and C-5'''), 128.1 (C-2'' and C-6''), 128.0 (C-2''' and C-6'''), 127.8 (C-4''), 127.1 (C-5), 126.5 (C-4'''), 124.5 (C-1), 123.6 (C-8), 121.1 (C-8a), 120.6 (C-9a), 118.9 (C-4), 74.9 (C-2'), 59.8 (C-1'), 20.8 (C7-CH_3), 15.7 (C5-CH_3).

2. Enantiomeric Purity

2.1. Instrumentation and chromatographic conditions

Liquid chromatography analysis was performed in Laboratory of Organic and Medicinal Chemistry of the Faculty of Pharmacy of the University of Porto. Analytical HPLC analyses were performed on a SpectraSYSTEM (Thermo Fisher Scientific, Inc, USA) equipped with a P4000 pump, an AS3000 autosampler and a diode array detector UV8000. The separation was carried out on (*S,S*)-Whelk-O1 CSP (150 x 4.6 mm i.d. column) obtained from Regis Technologies, Inc. (Morton Grove, Illinois, USA). Ethanol (EtOH), methanol (MeOH), acetonitrile (ACN) for HPLC were purchased from Sigma-Aldrich Co (St. Louis, Missouri, USA).

The polar organic mode LC evaluation was carried out using a mixture of MeOH and ACN. The mobile phase was prepared in a volume/volume (50:50 *v/v*) relation and degassed in an ultrasonic bath for 15 min before use. The flow rate used was 1 mL/min and the chromatograms were monitored by UV detection at a wavelength of 254 nm. The sample injections (25 µL) were carried out in triplicate. The dead time (t_0) was considered to be equal to the peak of the solvent front and was taken from each particular run. The stock solutions of CDXs in EtOH at the concentration of 1 mg/mL were prepared and further diluted in the same solvent to a concentration of 0.1 mg/mL. Working solutions of enantiomeric mixtures of CDXs were prepared mixing equal aliquots of each enantiomer. The elution order and e.e determinations were carried out with the stock solutions of each enantiomer of CDXs diluted at the concentration of 20 µg/mL. Solutions of 50 µg/mL of each enantiomer spiked with 0.5% of the opposite enantiomer were also prepared. The analyses were performed at room temperature.

The retention factor (k) was calculated using the equation ($k = [t_R - t_0] / t_0$). The separation factor (α) was calculated as ($\alpha = k_2/k_1$). The resolution factor (Rs) was calculated using the equation ($Rs = 1.18\ [t_{R2} - t_{R1}]/[W_{1\ 0.5}+W_{2\ 0.5}]$) where t_{R1} and t_{R2} are the retention times of the first and second enantiomers, respectively, and $W_{1\ 0.5}$ and $W_{2\ 0.5}$ are the corresponding peak width measured on half height.[111]

3. Biological activity

3.1. Cell Cultures

The three human tumor cell lines, A375-C5 (melanoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer), were grown as monolayer and routinely maintained in cell culture medium RPMI-1640 (with Glutamax, Lonza) supplemented with 5% heat-inactivated fetal bovine serum (FBS), and incubated in a humidified incubator at 37 °C with 5% CO_2 (Hera Cell, Heraeus). Cell number and viability were routinely determined with Trypan Blue (Sigma) exclusion assay. All experiments were performed with cells in exponential growth with viabilities over 90% and repeated at least three times.

3.2. Cell growth assay inhibitory assay

The effects of compounds on the growth of human tumor cell lines were evaluated according to the procedure adopted by the NCI in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B (SRB) to assess cell growth.[110] The optimal plating density of each cell line, that ensures exponential growth throughout all the experimental period, was 5 X 10^5 cells/mL for MCF-7 and NCI-H460 and 7.5 X 10^5 cells/mL for A375-C5. Cells in 96-well plates were allowed to attach overnight and then exposed for 48h to five dilutions, starting from maximum concentration of 150 μM. Following this incubation period, the adherent cells were fixed in situ, washed and stained with SRB. The bound stain was solubilized and the absorbance was measured at 515 ηm in a plate reader (Biotek Synergy 2). For each tested compound and for each cell line a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of compound that inhibited 50% of the net cell growth, was calculated as described.[110] Doxorubicin, used as a positive control, was tested in the same manner. The effect of the vehicle solvent (DMSO) was also evaluated by exposing untreated control cells to the maximum concentration of DMSO used in each assay (0.25%).

Chapter 4:

Conclusions

Currently, chirality is considered one of major driving forces in design, discovery, and development, launching and marketing of new drugs, especially when dealing with single enantiomeric drugs. Xanthones (9*H*-xanthon-9-ones) are privileged structures and many have proved to be important building blocks for synthesis of new compounds. Among them, chiral derivatives of xanthones (CDXs) represent an attractive area of Pharmaceutical Medicinal Chemistry research concerning their broad spectrum of biological/pharmacological activities.

This work described the synthesis of a library of twenty four CDXs in enantiomerically pure form by an efficient and mild methodology. The CDXs were synthesized by coupling two suitable functionalized xanthone derivatives as chemical substrates with a variety of commercially available chiral building blocks, namely amines and amino alcohols. The coupling reactions were carried out with the coupling reagent *O*-(benzotriazol-1-yl)-*N*-*N*-*N*'-*N*'-tetramethyluronium tetrafluoroborate (TBTU).

Three carboxyxanthone derivatives (XCars) were also successfully synthesized by a multi-step synthetic pathway, via diaryl ether intermediate (Ullman synthesis).

On the basis of our current state of knowledge all the synthesized CDXs and one of the XCar are describe here for the first time.

The evaluation of enantiomeric purity of all synthesised CDXs was determined by enantioselective LC method using the (*S*,*S*)-Whelk-O1 chiral stationary phase (CSP) under polar organic mode elution conditions.

Considering growth inhibitory effects on human tumor cell lines, some CDXs exhibited interesting results, with some cases of enantioselectivity. Furthermore, the nature and positions of substituents on the xanthonic scaffold of CDXs demonstrate to have great influence on the growth inhibitory effects.

Taking in consideration the results obtained and described in this work it can be saying that the main objectives of this dissertation were accomplished.

Chapter 5:

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The search for the references used in the present dissertation was made using the following browsers (last access in June of 2016).

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Chapter 6:

Appendixes

Table 24: Chemical structures and biological/pharmacological activities of CDXs.

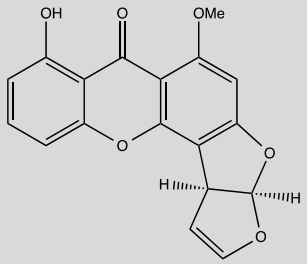
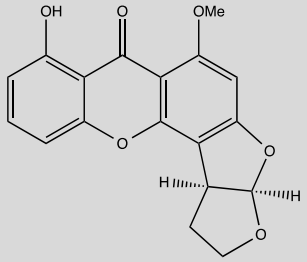
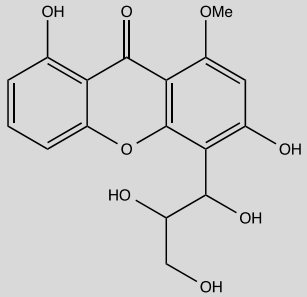
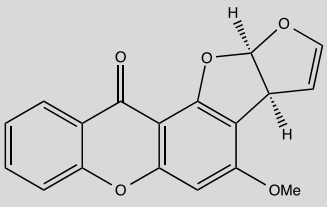
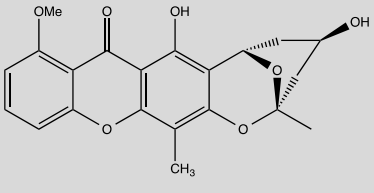
Structure	Name	Origin	Biological/ pharmacological activity
 **(1)**	Sterigmatocystin	Natural and Synthetic	Antitumor [50,51,53,54]
 **(2)**	Dihydrosterigmatocystin	Natural and Synthetic	Antitumor [50,52,53]
 **(3)**	Secosterigmatocystin	Natural and Synthetic	Antitumor [50,52,53]
 **(4)**	Asperxanthone	Natural and Synthetic	Tabacco mosaic virus inhibition [50,55]
 **(5)**	Chaetoxanthone A	Natural and Synthetic	Antiprotozoal [50,56]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

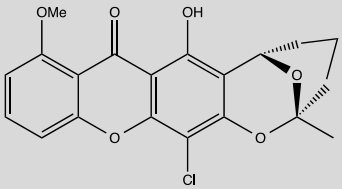
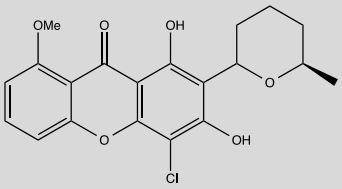
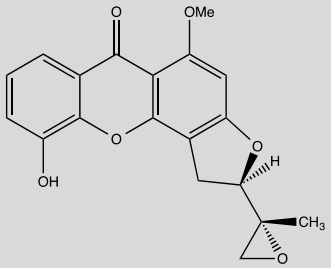
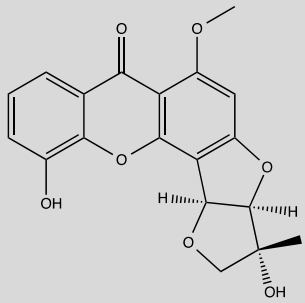
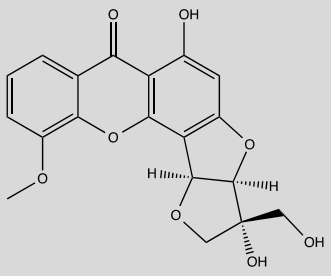
Structure	Name	Origin	Biological/ pharmacological activity
 **(6)**	Chaetoxanthone B	Natural and Synthetic	Antiprotozoal [50,56]
 **(7)**	Chaetoxanthone C	Natural and Synthetic	Antiprotozoal [50,56]
 **(8)**	Psorospermin	Natural and Synthetic	Antitumor [39,57,58,60]
 **(9)**	Psorofebrin	Natural and Synthetic	Antitumor [60]
 **(10)**	Isohydroxyisopsorofebrin	Natural and Synthetic	Antitumor [60]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

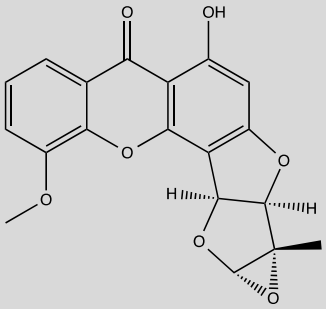
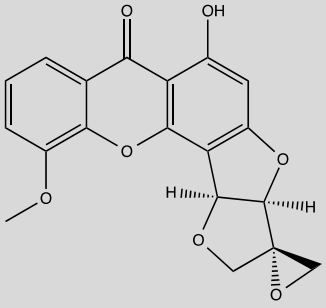
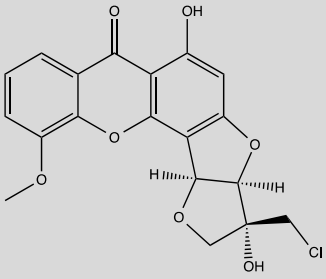
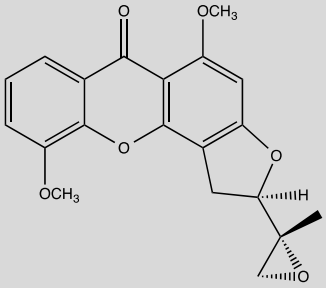
Structure	Name	Origin	Biological/ pharmacological activity
 **(11)**	(8a*R*,8b*S*,9a*S*,10a*S*)-6-hydroxy-1-methoxy-8b-methyl-8a,8b,9a,10a-tetrahydro-5*H*-oxireno[2'',3'':4',5']furo[2',3':4,5]furo[2,3-*c*]xanthen-5-one	Natural and Synthetic	Antitumor [60]
 **(12)**	(3*R*,3a*R*,12c*S*)-6-hydroxy-11-methoxy-3a,12c-dihydro-2*H*,7*H*-spiro[furo[2',3':4,5]furo[2,3-*c*]xanthene-3,2'-oxiran]-7-one	Natural and Synthetic	Antitumor [60]
 **(13)**	Chlorohydrin	Natural and Synthetic	Antitumor [60]
 **(14)**	(2*R*,3*R*) O-5-methyl psorospermin	Natural and Synthetic	Antitumor [61]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

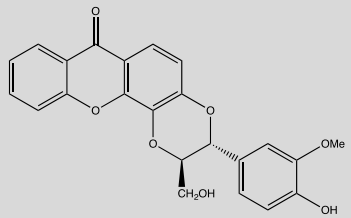
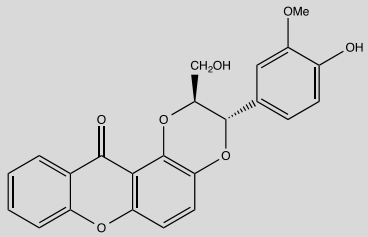
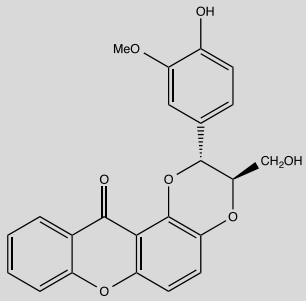
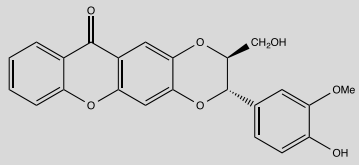
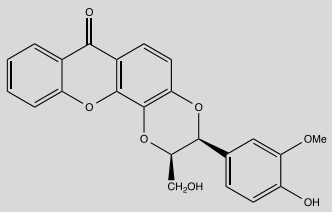
Structure	Name	Origin	Biological/ pharmacological activity
 **(15)**	(2*R*,3*R*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-7*H*-[1,4]dioxino[2,3-c]xanthen-7-one	Synthetic	Antitumor, PKC and cellular proliferation inhibition [39,65]
 **(16)**	(2*S*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-12*H*-[1,4]dioxino[2,3-a]xanthen-12-one	Synthetic	Antitumor, PKC and cellular proliferation inhibition [39,65]
 **(17)**	(2*R*,3*R*)-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-12*H*-[1,4]dioxino[2,3-a]xanthen-12-one	Synthetic	Antitumor, PKC and cellular proliferation inhibition [39,65]
 **(18)**	(2*S*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-11*H*-[1,4]dioxino[2,3-b]xanthen-11-one	Synthetic	Antitumor, PKC and cellular proliferation inhibition [39,65]
 **(19)**	(2*R*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydro-7*H*-[1,4]dioxino[2,3-c]xanthen-7-one	Synthetic	Antitumor, PKC and cellular proliferation inhibition [39,65]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

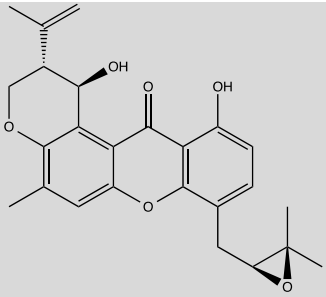
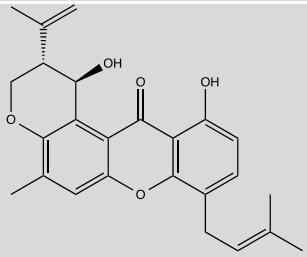
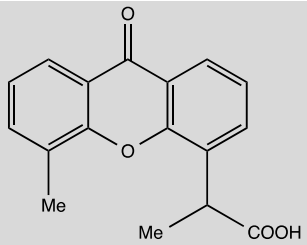
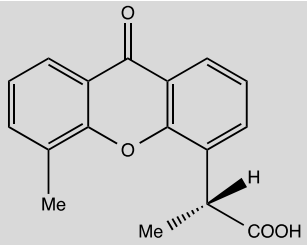
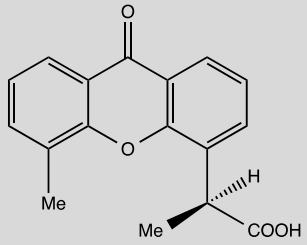
Structure	Name	Origin	Biological/ pharmacological activity
 **(20)**	Tajixanthone	Natural and Synthetic	Antitumor [50,68-71]
 **(21)**	Shamixanthone	Natural and Synthetic	Antitumor [50,68-71]
 **(22)**	2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid	Synthetic	Antitumor [74]
 **(22a)**	(*R*)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid	Synthetic	Antitumor [74]
 **(22b)**	(*S*)-2-(5-methyl-9-oxo-9*H*-xanthen-4-yl)propanoic acid	Synthetic	Antitumor [74]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

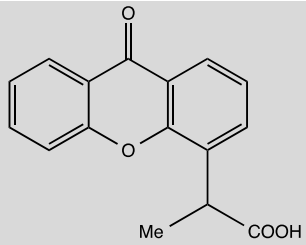
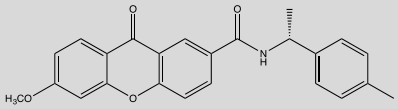
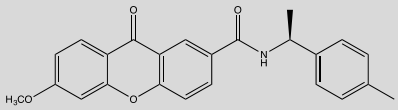
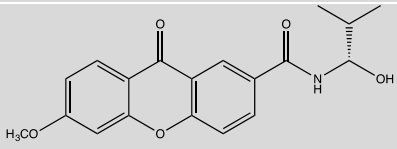
Structure	Name	Origin	Biological/ pharmacological activity
 **(23)**	2-(9-oxo-9*H*-xanthen-4-yl)propanoic acid	Synthetic	Antitumor and Antiinflammatory [74]
 **(24a)** **e.e. (%) > 99 %** **Column amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λ_{max} 254 nm) [29].**	(*R*)-6-methoxy-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(24b)** **e.e. (%) > 99%** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λ_{max} 254 nm) [29]**	(*S*)-6-methoxy-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide	Synthetic	Local anaesthetic, and growth of human tumor cell lines inhibition [29,77,80]
 **(25a)** **e.e. (%) > 99 %** **Column: Chirobiotic T, Mobile phase: n-hexane/EtOH (80:20 *v:v*), 0.5 mL/min, λmax 254 nm) [29]**	(*R*)-N-(1-hydroxy-3-methylbutan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

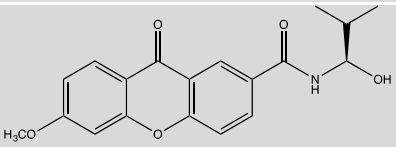
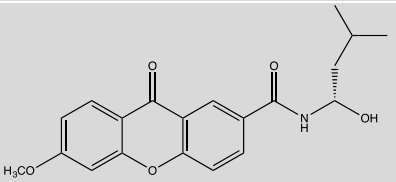
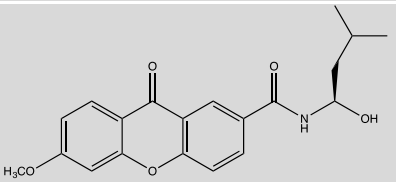
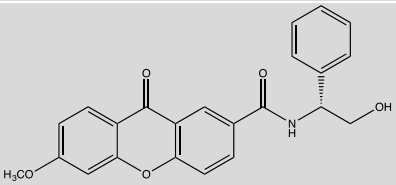
Structure	Name	Origin	Biological/ pharmacological activity
 **(25b)** **e.e. (%) > 99 %** **Column: Chirobiotic T, Mobile phase: n-hexane/EtOH (80:20 *v:v*), 0.5 mL/min, λmax 254 nm) [29].**	(*S)*-N-(1-hydroxy-3-methylbutan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Local anaesthetic, and growth of human tumor cell lines inhibition [29,77,80]
 **(26a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λmax 254 nm)[29]**	(*R*)-N-(1-hydroxy-4-methylpentan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(26b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λmax 254 nm) [29]**	(*S*)-N-(1-hydroxy-4-methylpentan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Local anaesthetic, and growth of human tumor cell lines inhibition [29,77,80]
 **(27a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*)[29]**	(*R*)-N-(2-hydroxy-1-phenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

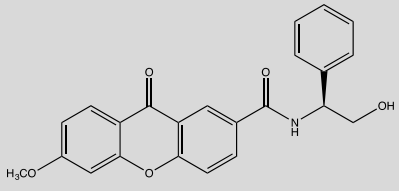
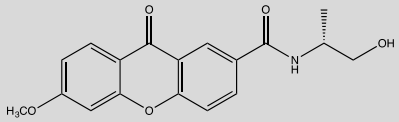
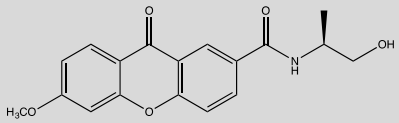
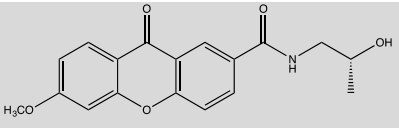
Structure	Name	Origin	Biological/ pharmacological activity
 **(27b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λmax 254 nm) [29].**	(*S*)-N-(2-hydroxy-1-phenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(28a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λmax 254 nm)**	(*R*)-N-(1-hydroxypropan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(28b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: EtOH/ACN (50:50 *v:v*), 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(1-hydroxypropan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(29a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: n-hexane/2-PrOH (50:50 *v:v*), 0.5mL/min, λmax 254 nm)[29].**	(*R*)-N-(2-hydroxypropyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

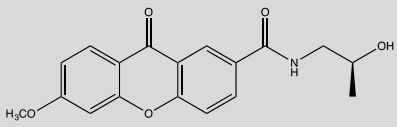
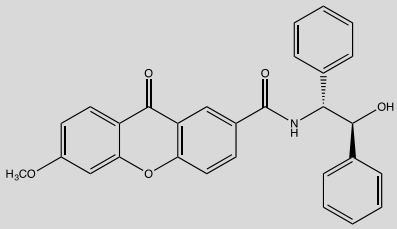
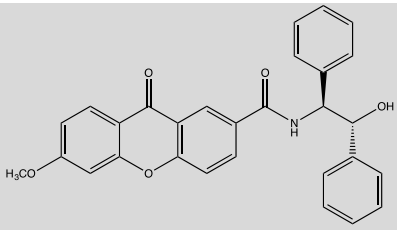
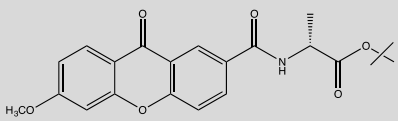
Structure	Name	Origin	Biological/ pharmacological activity
 **(29b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: n-hexane/ 2-PrOH (50:50 *v:v*), 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(2-hydroxypropyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(30c)** **e.e. (%) > 97 %** **Column: (S,S)-Whelk-O1, Mobile phase: ACN/ MeOH (50:50 *v:v*), 1.0 mL/min, λmax 254 nm)[29].**	N-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(30d)** **e.e. (%) > 98 %** **Column: (S,S)-Whelk-O1, Mobile phase: ACN/MeOH (50:50 *v:v*), 1.0 mL/min, λmax 254 nm)[29].**	N-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(31a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: MeOH, 0.5 mL/min, λmax 254 nm)[29]**	(*R*)-tert-butyl 2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido) propanoate	Synthetic	Growth of human tumor cell lines inhibition [29]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

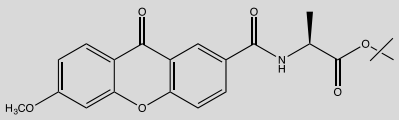
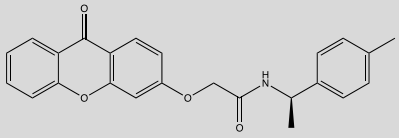
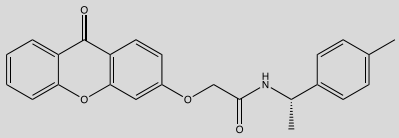
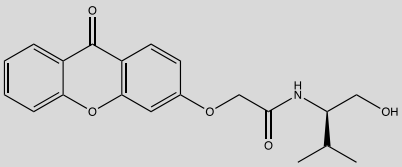
Structure	Name	Origin	Biological/ pharmacological activity
 **(31b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: MeOH 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-tert-butyl 2-(6-methoxy-9-oxo-9*H*-xanthene-2-carboxamido) propanoate	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(32a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucle- osil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*R*)-2-((9-oxo-9*H*-xanthen-3-yl)oxy)-N-(1-(p-tolyl)ethyl) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(32b)** **e.e. (%) > 98 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-2-((9-oxo-9*H*-xanthen-3-yl)oxy)-N-(1-(p-tolyl)ethyl) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(33a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: MeOH, 0.5 mL/min, λmax 254 nm)[29].**	(*R*)-N-(1-hydroxy-3-methylbutan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

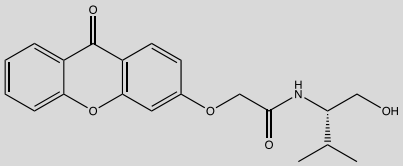
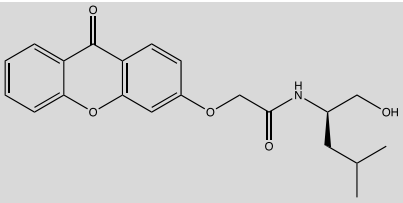
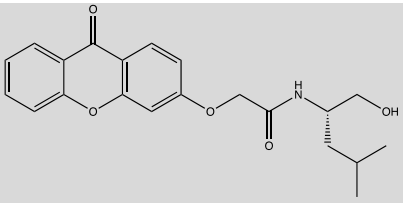
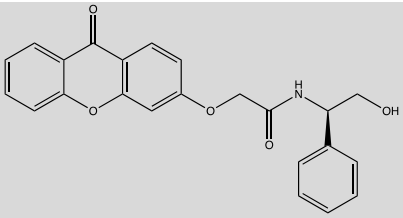
Structure	Name	Origin	Biological/ pharmacological activity
 **(33b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: MeOH, 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(1-hydroxy-3-methylbutan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(34a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN 0.5 mL/min, λmax 254 nm)[29].**	(*R*)-N-(1-hydroxy-4-methylpentan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(34b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(1-hydroxy-4-methylpentan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(35a)** **e.e. (%) > 99 %** **Column: cellulose tris-3,5-dimethyl-phenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% w/ w), Mobile phase: n-hexane/EtOH (70:30 v:v), 0.5 mL/min, λmax 254 nm)[29]**	(*R*)-N-(2-hydroxy-1-phenylethyl)-2-((9-oxo-9H-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

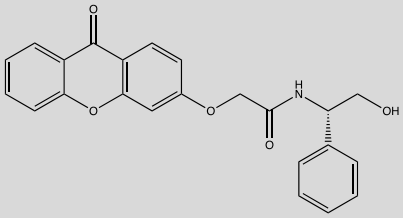
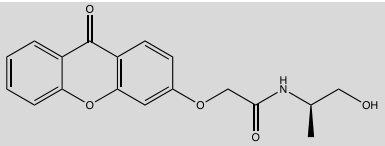
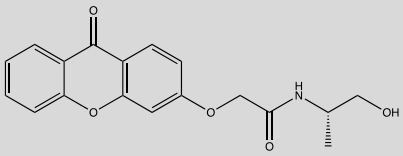
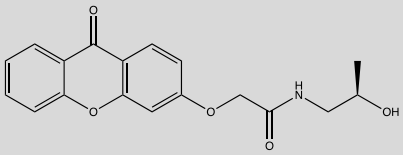
Structure	Name	Origin	Biological/ pharmacological activity
 **(35b)** **e.e. (%) > 99 %** **Column: cellulose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: n-hexane/ EtOH (70:30 *v:v*), 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(2-hydroxy-1-phenylethyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29,80]
 **(36a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarba- mate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*R*)-N-(1-hydroxypropan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(36b)** **e.e. (%) >99 %** **Column: amylose tris-3,5-dimethylphenylcarba- mate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(1-hydroxypropan-2-yl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(37a)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*R*)-N-(2-hydroxypropyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

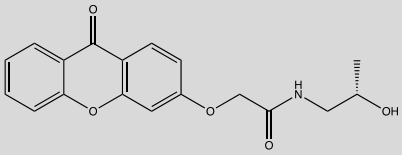
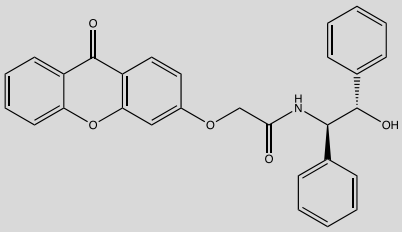
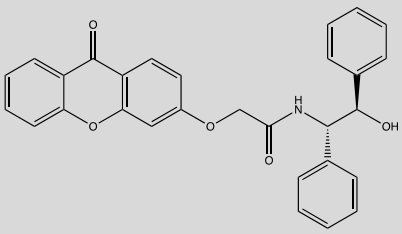
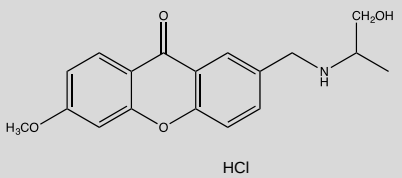
Structure	Name	Origin	Biological/ pharmacological activity
 **(37b)** **e.e. (%) > 99 %** **Column: amylose tris-3,5-dimethylphenylcarbamate coated onto APS-Nucleosil (500 Å, 7 lm, 20% *w/w*), Mobile phase: ACN, 0.5 mL/min, λmax 254 nm)[29].**	(*S*)-N-(2-hydroxypropyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(38c)** **e.e. (%) > 98 %** **Column: (*S,S*)- Whelk-O1, Mobile phase: ACN/MeOH (50:50 *v:v*), 1.0 mL/min, kmax 254 nm)[29].**	*N*-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 **(38d)** **e.e. (%) > 99 %** **Column: (*S,S*)-Whelk-O1, Mobile phase: ACN/MeOH (50:50 *v:v*), 1.0 mL/min, λmax 254 nm)[29].**	*N*-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy) acetamide	Synthetic	Growth of human tumor cell lines inhibition [29]
 HCl **(39)**	2-(((1-hydroxypropan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiplatelets, Antimutagenic and Antiarrhythmic [40,91,96,97]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

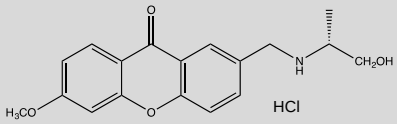
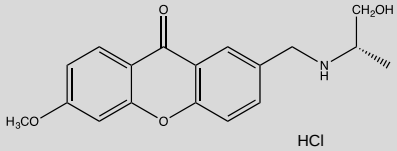
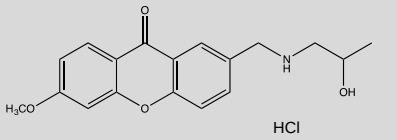
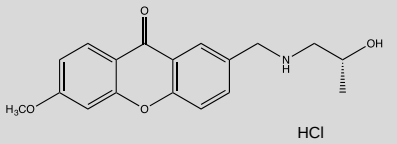
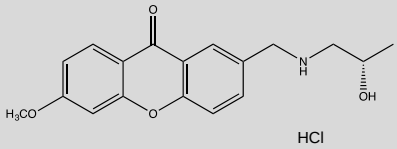
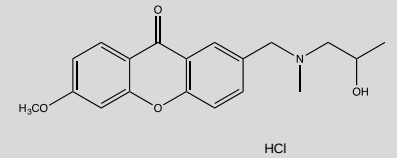
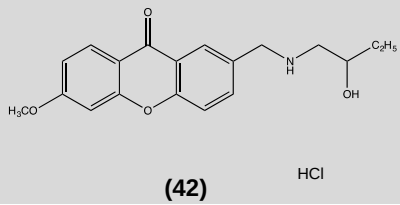
Structure	Name	Origin	Biological/ pharmacological activity
 **(39a)**	(*R*)-2-(((1-hydroxypropan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40,89]
 **(39b)**	(*S*)-2-(((1-hydroxypropan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(40)**	2-(((2-hydroxypropyl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiplatelets [40,89,91,96]
 **(40a)**	(*R*)-2-(((2-hydroxypropyl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(40b)**	(*S*)-2-(((2-hydroxypropyl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(41)**	2-(((2-hydroxypropyl)(methyl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiplatelets [40,91,96]
 **(42)**	2-(((2-hydroxybutyl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40,97]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

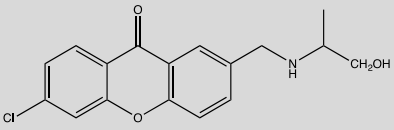
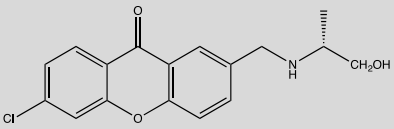
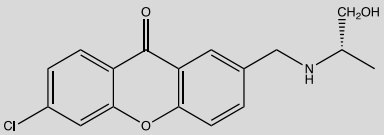
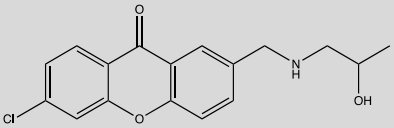
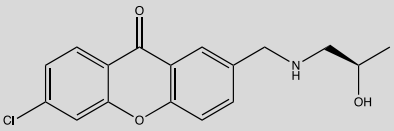
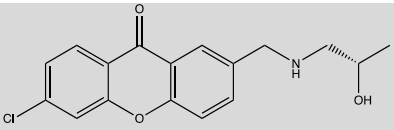
Structure	Name	Origin	Biological/ pharmacological activity
 **(43)**	6-chloro-2-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant and Antimutagenic [40,92,97]
 **(43a)** **e.e. (%) > 99,9 %**	(*R*)-6-chloro-2-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [85,92]
 **(43b)** **e.e. (%) = > 99,0%**	(*S*)-6-chloro-2-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [85,92]
 **(44)**	6-chloro-2-(((2-hydroxypropyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(44a)**	(*R*)-6-chloro-2-(((2-hydroxypropyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [85]
 **(44b)**	(*S*)-6-chloro-2-(((2-hydroxypropyl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [85]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

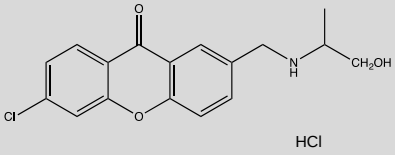
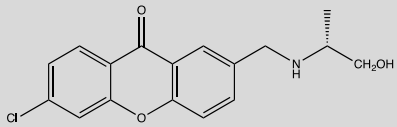
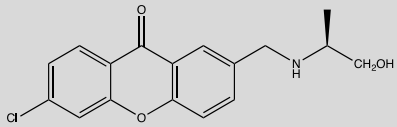
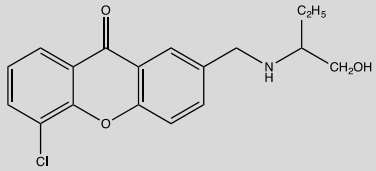
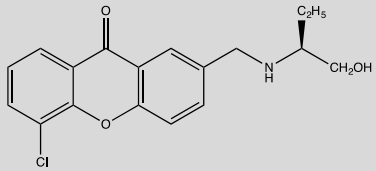
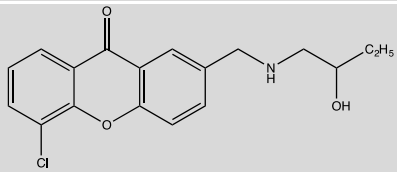
Structure	Name	Origin	Biological/ pharmacological activity
 **(45)**	6-chloro-2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [40,93]
 **(45a)**	(*R*)-6-chloro-2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [85,93]
 **(45b)**	(*S*)-6-chloro-2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity
 **(46)**	5-chloro-2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(46b)**	(*S*)-5-chloro-2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [40]
 **(47)**	2-(((2-hydroxybutyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [83]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

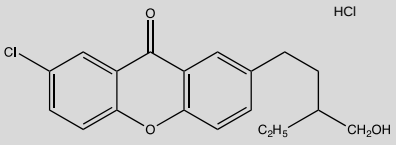
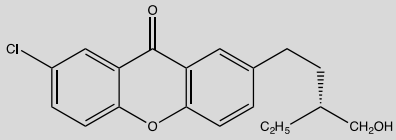
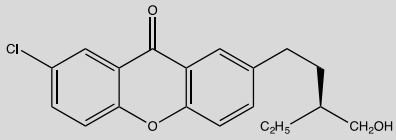
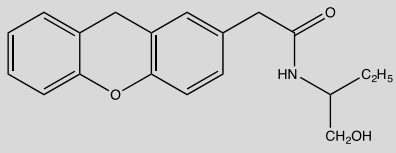
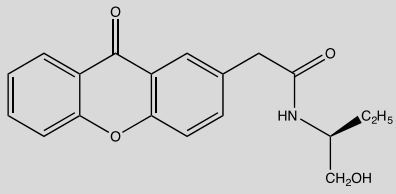
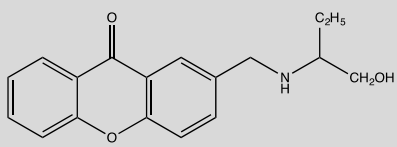
Structure	Name	Origin	Biological/ pharmacological activity
 **(48)**	2-chloro-7-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [83,93]
 **(48a)** HCl	(*R*)-2-chloro-7-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [83,93]
 **(48b)**	(*S*)-2-chloro-7-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [83,93]
 **(49)**	*N*-(1-hydroxybutan-2-yl)-2-(9-oxo-9*H*-xanthen-2-yl)acetamide	Synthetic	Anticonvulsant
 **(50)**	(*S*)-*N*-(1-hydroxybutan-2-yl)-2-(9-oxo-9*H*-xanthen-2-yl)acetamide	Synthetic	Anticonvulsant [83]
 **(51)**	2-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [83]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

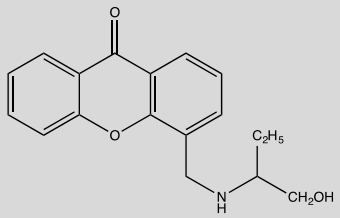
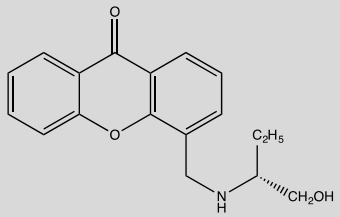
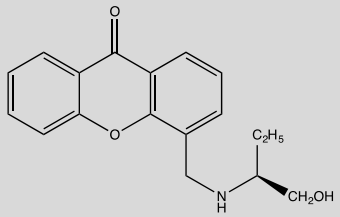
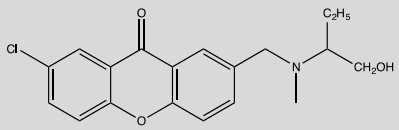
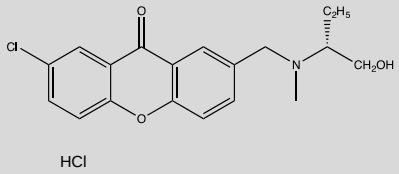
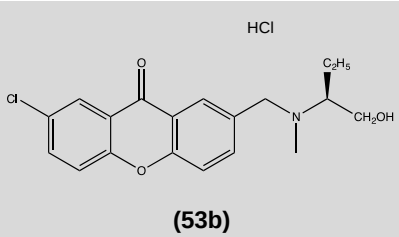
Structure	Name	Origin	Biological/ pharmacological activity
 **(52)**	4-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic [93]
 **(52a)**	(*R*)-4-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [93]
 **(52b)**	(*S*)-4-(((1-hydroxybutan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Local anesthetic and Hemodynamic activity [93]
 **(53)**	2-chloro-7-(((1-hydroxybutan-2-yl)(methyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [83,93]
 **(53a)**	(*R*)-2-chloro-7-(((1-hydroxybutan-2-yl)(methyl)amino)methyl)-9H-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiplatelets [83,91,93]
 **(53b)**	(*S*)-2-chloro-7-(((1-hydroxybutan-2-yl)(methyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [83,93]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

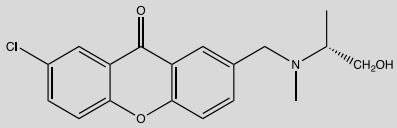
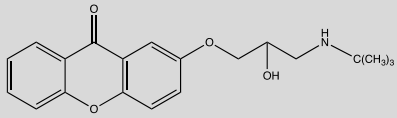
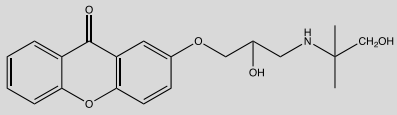
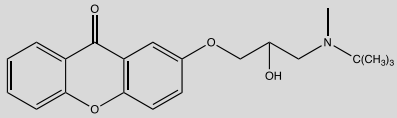
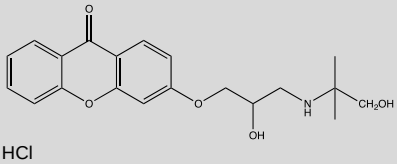
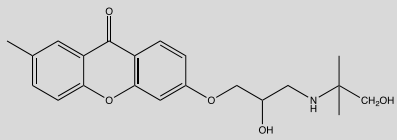
Structure	Name	Origin	Biological/ pharmacological activity
 HCl **(54a)**	(*R*)-2-chloro-7-(((1-hydroxypropan-2-yl)(methyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [91]
 HCl **(55)**	2-(3-(tert-butylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antipiletic, Antiplatelets and Antimycobacterial [84,91,96,99]
 HCl **(56)**	2-(2-hydroxy-3-((1-hydroxy-2-methylpropan-2-yl)amino)propoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antipiletic and Antiplatelets [84,86,96]
 **(57)**	2-(3-(tert-butyl(methyl)amino)-2-hydroxypropoxy)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 HCl **(58)**	3-(2-hydroxy-3-((1-hydroxy-2-methylpropan-2-yl)amino)propoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiarrhythmic [86]
 HCl **(59)**	6-(2-hydroxy-3-((1-hydroxy-2-methylpropan-2-yl)amino)propoxy)-2-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiarrhythmic [86]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

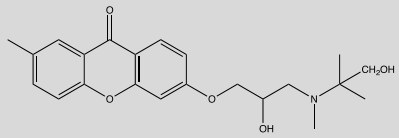
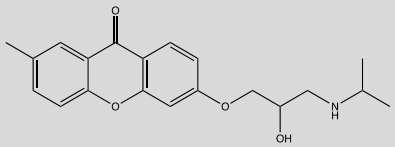
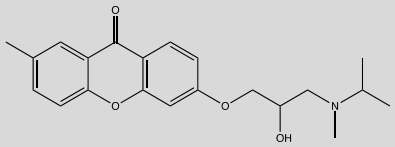
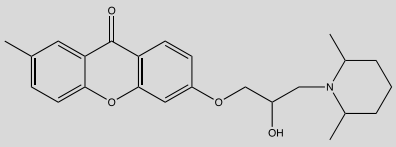
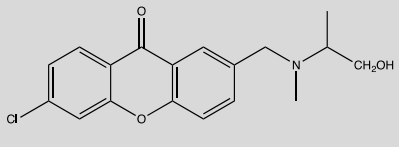
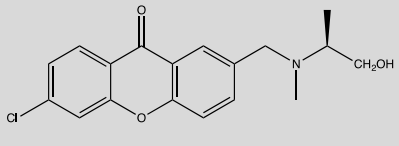
Structure	Name	Origin	Biological/ pharmacological activity
 HCl **(60)**	6-(2-hydroxy-3-((1-hydroxy-2-methylpropan-2-yl)(methyl)amino)propoxy)-2-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiarrhythmic [86]
 **(61)**	6-(2-hydroxy-3-(isopropylamino)propoxy)-2-methyl-9*H*-xanthen-9-one	Synthetic	Anticonvulsant and Antimutagenic [86,97]
 HCl **(62)**	6-(2-hydroxy-3-(isopropyl(methyl)amino)propoxy)-2-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [86]
 HCl **(63)**	6-(3-(2,6-dimethylpiperidin-1-yl)-2-hydroxypropoxy)-2-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [86]
 HCl **(64)**	6-chloro-2-(((1-hydroxypropan-2-yl)(methyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antimutagenic [89,97]
 HCl **(64b)**	(*S*)-6-chloro-2-(((1-hydroxypropan-2-yl)(methyl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [85]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

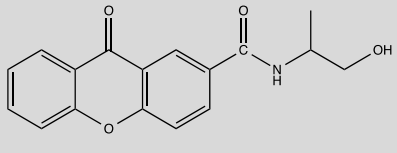
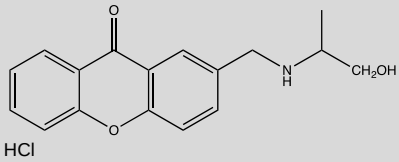
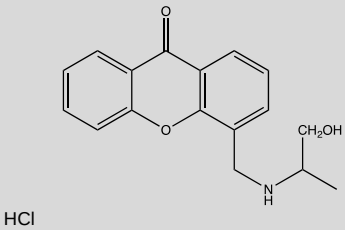
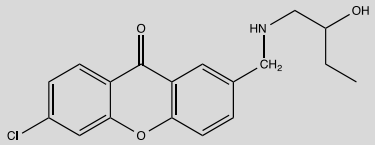
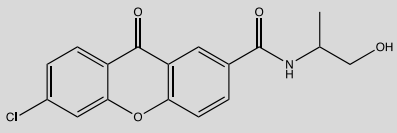
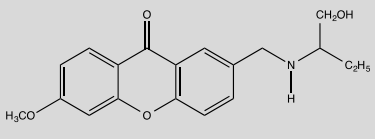
Structure	Name	Origin	Biological/ pharmacological activity
 **(65)**	*N*-(1-hydroxypropan-2-yl)-9-oxo-9*H*-xanthene-2-carboxamide	Synthetic	Anticonvulsant [85]
 HCl **(66)**	2-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiarrhythmic [89]
 HCl **(67)**	4-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant and Antiarrhythmic [89]
 **(68)**	6-Chloro-2-((2-hydroxybutylamino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [85]
 **(69)**	6-Chloro-*N*-(1-hydroxypropan-2-yl)-9-oxo-9*H*-xanthene-2-carboxiamide	Synthetic	Anticonvulsant [85]
 **(70)**	2-(((1-hydroxybutan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90,93]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

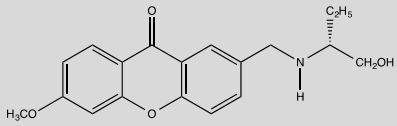
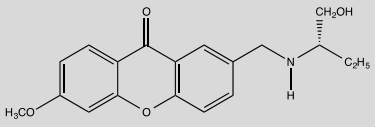
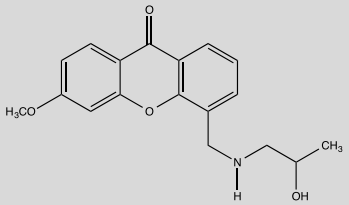
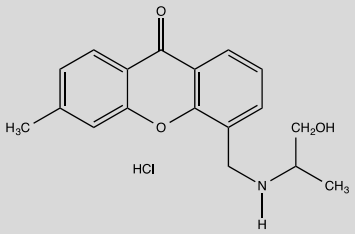
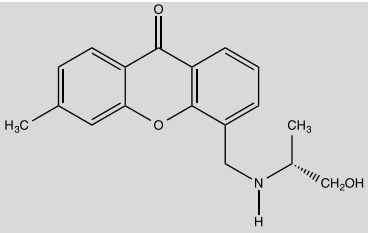
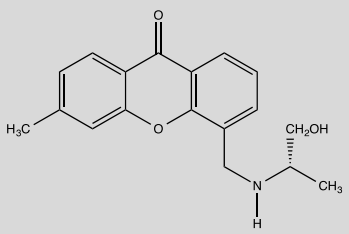
Structure	Name	Origin	Biological/ pharmacological activity
 **(70a)**	(*R*)-2-(((1-hydroxybutan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90,93]
 **(70b)**	(*S*)-2-(((1-hydroxybutan-2-yl)amino)methyl)-6-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90,93]
 **(71)**	5-(((2-hydroxypropyl)amino)methyl)-3-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(72)**	5-(((1-hydroxypropan-2-yl)amino)methyl)-3-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [90]
 **(72a)**	(*R*)-5-(((1-hydroxypropan-2-yl)amino)methyl)-3-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [90]
 **(72b)**	(*S*)-5-(((1-hydroxypropan-2-yl)amino)methyl)-3-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [90]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

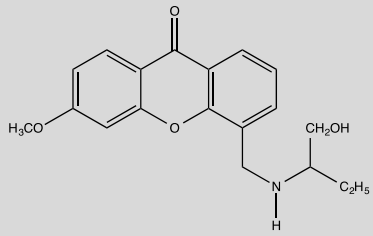
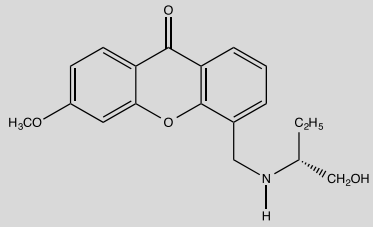
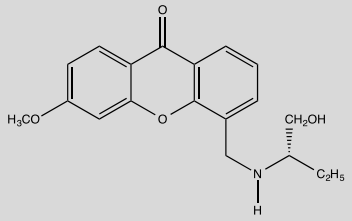
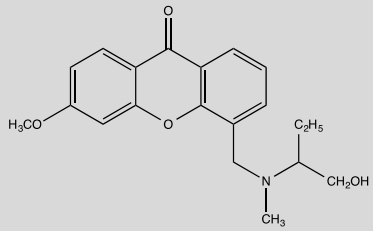
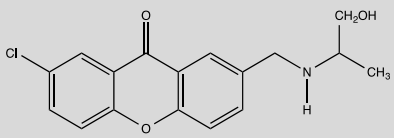
Structure	Name	Origin	Biological/ pharmacological activity
 **(73)**	5-(((1-hydroxybutan-2-yl)amino)methyl)-3-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(73a)**	(*R*)-5-(((1-hydroxybutan-2-yl)amino)methyl)-3-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(73b)**	(*S*)-5-(((1-hydroxybutan-2-yl)amino)methyl)-3-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(74)**	5-(((1-hydroxybutan-2-yl)(methyl)amino)methyl)-3-methoxy-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(75)**	2-chloro-7-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

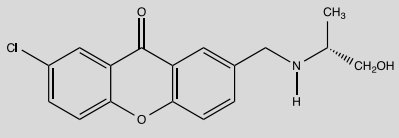
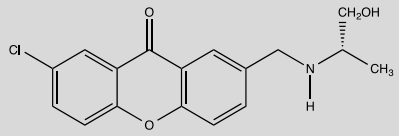
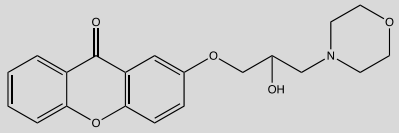
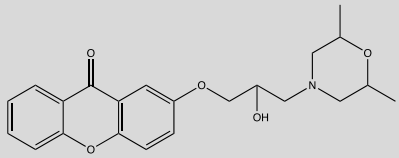
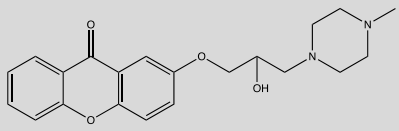
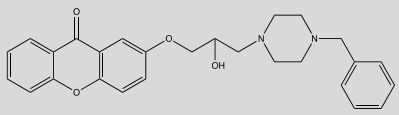
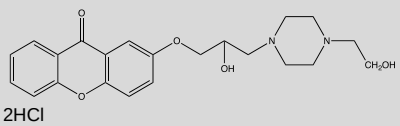
Structure	Name	Origin	Biological/ pharmacological activity
 **(75a)**	(*R*)-2-chloro-7-(((1-hydroxypropan-2-yl)amino)methyl)-9H-xanthen-9-one	Synthetic	Anticonvulsant [90]
 **(75b)**	(*S*)-2-chloro-7-(((1-hydroxypropan-2-yl)amino)methyl)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant [90]
 2 HCl **(76)**	2-(2-hydroxy-3-morpholinopropoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiplatelets and Antimycobacterial [96,99]
 HCl **(77)**	2-(3-(2,6-dimethylmorpholino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Antiplatelets and Antimycobacterial [96,99]
 HCl **(78)**	2-(2-hydroxy-3-(4-methylpiperazin-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 2HCl **(79)**	2-(3-(4-benzylpiperazin-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 2HCl **(80)**	2-(2-hydroxy-3-(4-(2-hydroxyethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Anticonvulsant, Antipiletic, Antiplatelets and Antimycobacterial [84,96,99]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

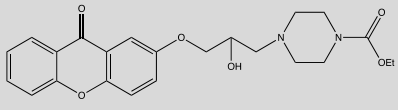
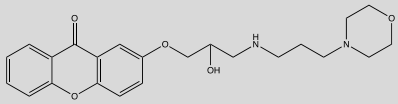
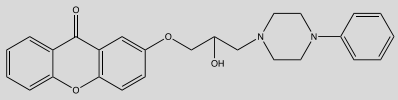
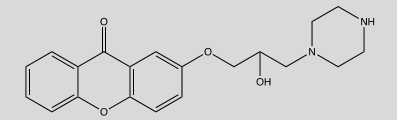
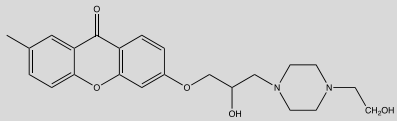
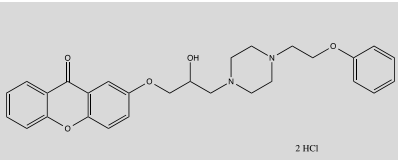
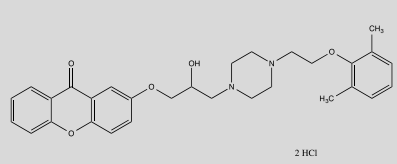
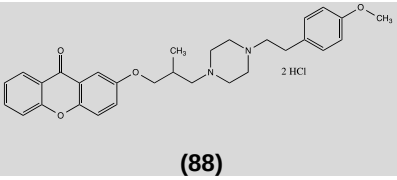
Structure	Name	Origin	Biological/ pharmacological activity
 **(81)**	Ethyl 4-(2-hydroxy-3-((9-oxo-9*H*-xanthen-2yl)oxy)propyl)piperazine-1-carboxylate	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 2HCl **(82)**	2-(2-hydroxy-3-((3-morpholinopropyl)amino) propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Anticonvulsant, Antipiletic And Antiplatelets [84,86,96]
 **(83)**	2-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propoxy)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 **(84)**	2-(2-hydroxy-3-(piperazin-1-yl)propoxy)-9*H*-xanthen-9-one	Synthetic	Anticonvulsant, Antipiletic and Antimycobacterial [84,99]
 HCl **(85)**	6-(2-hydroxy-3-(4-(2-hydroxyethyl)piperazin-1-yl)propoxy)-2-methyl-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant [86]
 **(86)**	2-(2hydroxy-3-(4-(2-phenoxyethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic and Antihypertensive [98]
 **(87)**	2-(3-(4-(2-(2,6-dimethylphenoxy)ethyl)piperazin-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic [98]
 **(88)**	2-(2-hydroxy-3-(4-(4-methoxyphenethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic [98]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

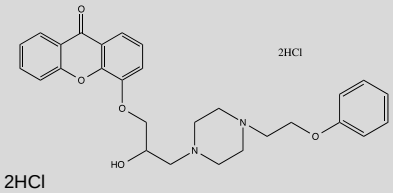
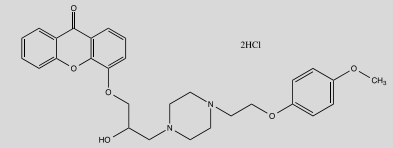
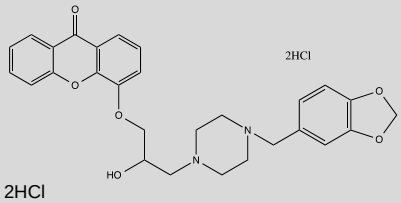
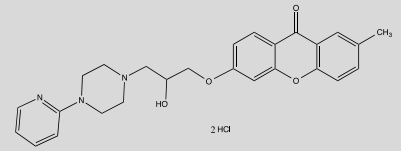
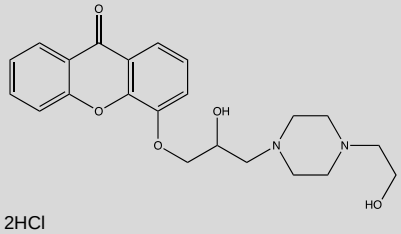
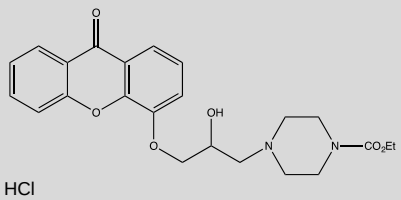
Structure	Name	Origin	Biological/ pharmacological activity
 **(89)**	4-(2-hydroxy-3-(4-(2-phenoxyethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic and Antihypertensive [98]
 **(90)**	4-(2-hydroxy-3-(4-(2-(4-methoxyphenoxy)ethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic [98]
 **(91)**	4-(3-(4-(benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic [98]
 **(92)**	6-(2-hydroxy-3-(4-(pyridin-2-yl)piperazin-1-yl)propoxy)-2-methyl-9*H*-xanthen-9-one dihydrochloride	Synthetic	Arrhythmic [98]
 **(93)**	4-(2-hydroxy-3-(4-(2-hydroxyethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiarrhythmic, and Antihypertensive [41]
 **(94)**	Ethyl 4-(2-hydroxy-3-((9-oxo-9*H*-xanthen-4-yl)oxy)propyl)piperazine-1-carboxylate hydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [41]

Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

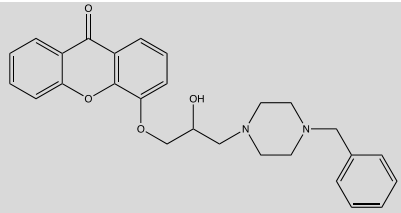
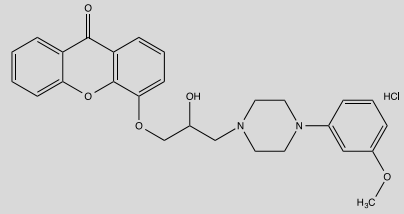
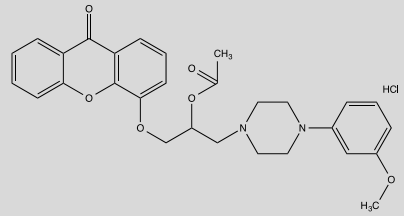
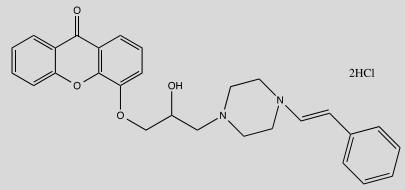
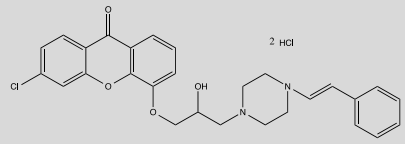
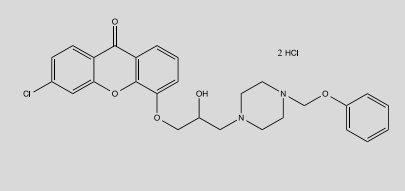
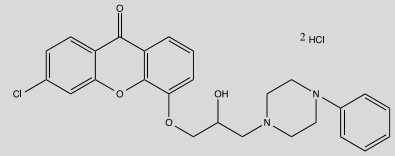
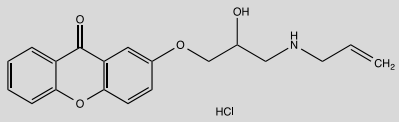
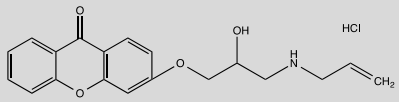
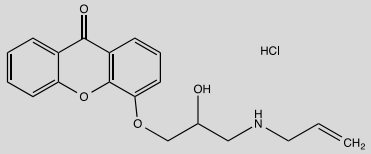
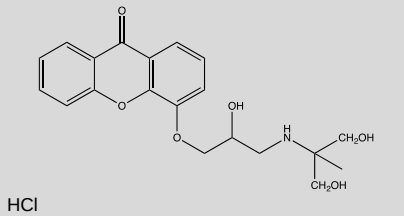
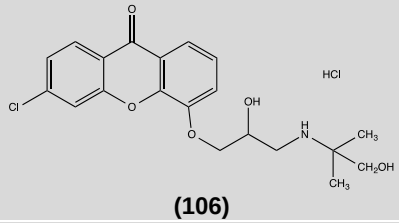
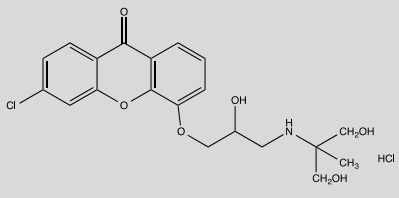
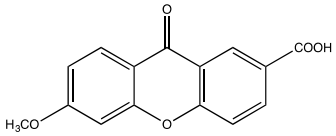
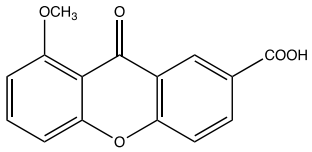
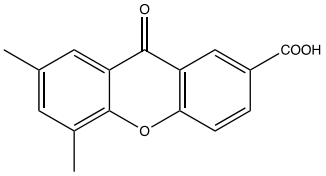
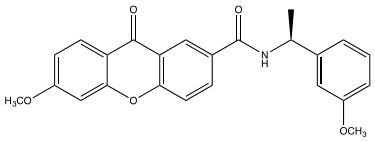
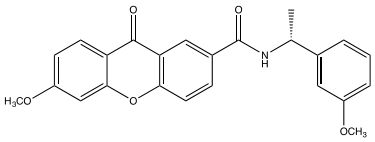
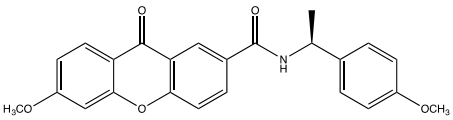
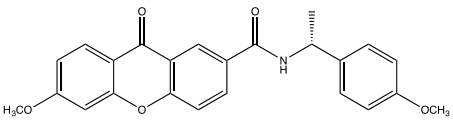
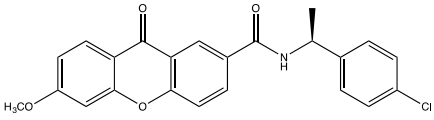
Structure	Name	Origin	Biological/ pharmacological activity
 2HCl **(95)**	4-(3-(4-benzylpiperazin-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [41]
 **(96)**	4-(2-hydroxy-3-(4-(3-methoxyphenyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]
 **(97)**	1-(4-(3-methoxyphenyl)piperazin-1-yl)-3-((9-oxo-9*H*-xanthen-4-yl)oxy)propan-2-yl acetate hydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]
 **(98)**	4-(2-hydroxy-3-(4-styrylpiperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]
 **(99)**	3-chloro-5-(2-hydroxy-3-(4-styrylpiperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]
 **(100)**	3-chloro-5-(2-hydroxy-3-(4-(phenoxymethyl)piperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]

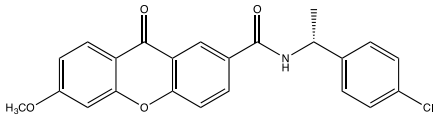
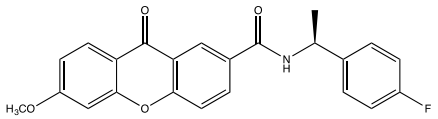
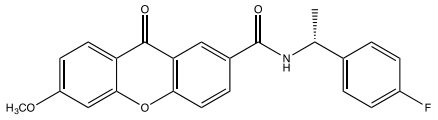
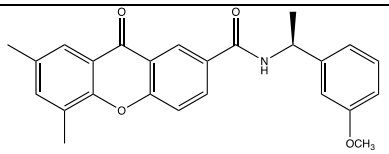
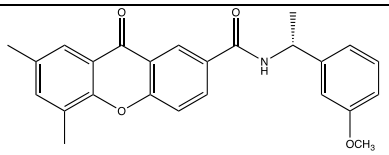
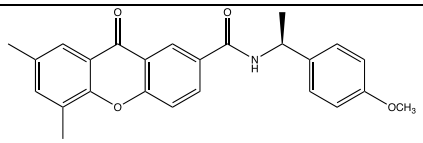
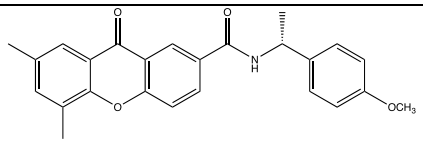
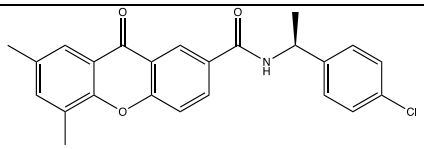
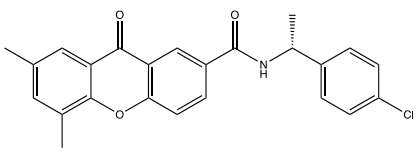
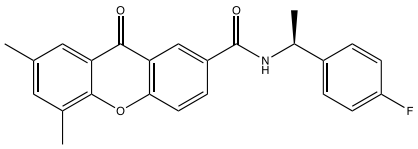
Table 24: Chemical structures and biological/pharmacological activities of CDXs (Continuation)

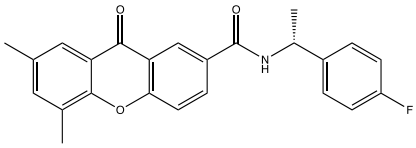
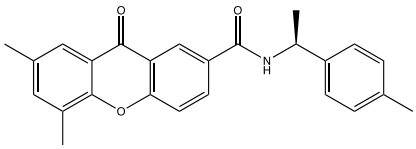
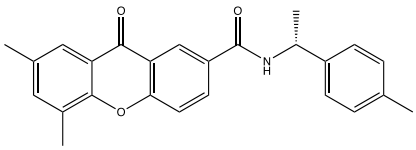
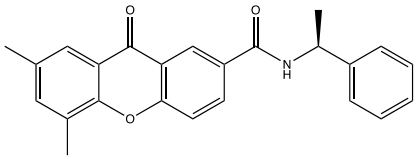
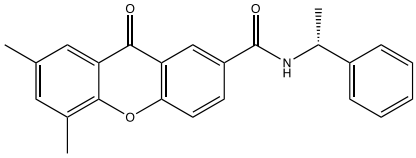
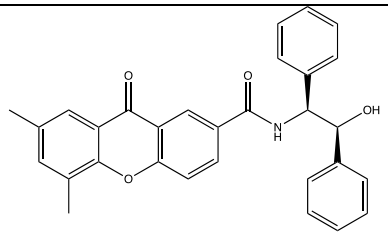
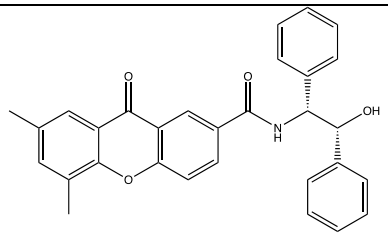
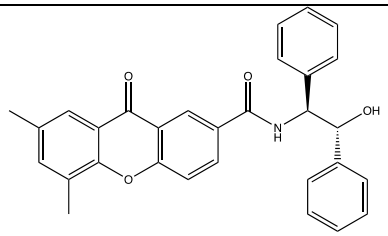
Structure	Name	Origin	Biological/ pharmacological activity
 **(101)**	3-chloro-5-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride	Synthetic	Antiarrhythmic and Antihypertensive [100]
 **(102)**	2-(3-(allylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic, Antihypertensive and Antifungal [87,88]
 **(103)**	3-(3-(allylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [88]
 **(104)**	4-(3-(allylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [88]
 **(105)**	4-(3-((1,3-Dihydroxy-2-methylpropan-2-yl)amino)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [88]
 **(106)**	3-Chloro-5-(2-hydroxy-3-((1-hydroxy-2-methylpropan-2-yl)amino)propoxy)-9H-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [88]
 **(107)**	3-Chloro-5-(3-((1,3-dihydroxy-2-methylpropan-2-yl)amino)-2-hydroxypropoxy)-9H-xanthen-9-one hydrochloride	Synthetic	Anticonvulsant, Antiarrhythmic and Antihypertensive [88]

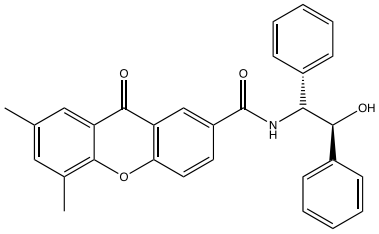
(a)–(*R*)-enantiomer; (b)–(*S*)-enantiomer; (c)–(*R,S*)-enantiomer; (d)–(*S,R*)- enantiomer.

Table 25: Structures of carboxyxanthones derivatives and chiral derivatives of xanthones

Structure	Name	Code
	6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid	XCar – 2 (108)
	8-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid	XCar-3 (109)
	5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxylic acid	XCar- 5 (110)
	(*S*)-6-methoxy-2-(3-(3-methoxyphenyl)butanoyl)-9*H*-xanthen-9-one	(*S*)-XEA-3-MET (137)
	(*R*)-6-methoxy-2-(3-(3-methoxyphenyl)butanoyl)-9*H*-xanthen-9-one	(*R*)-XEA-3-MET (138)
	(*S*)-6-methoxy-2-(3-(4-methoxyphenyl)butanoyl)-9*H*-xanthen-9-one	(*S*)-XEA-4-MET (139)
	(*R*)-6-methoxy-2-(3-(4-methoxyphenyl)butanoyl)-9*H*-xanthen-9-one	(*R*)-XEA-4-MET (140)
	(*S*)-2-(3-(4-chlorophenyl)butanoyl)-6-methoxy-9*H*-xanthen-9-one	(*S*)-XEA-4-CLO (141)

	(*R*)-2-(3-(4-chlorophenyl)butanoyl)-6-methoxy-9*H*-xanthen-9-one	(*R*)-XEA-4-CLO (142)
	(*S*)-2-(3-(4-fluorophenyl)butanoyl)-6-methoxy-9*H*-xanthen-9-one	(*S*)-XEA-4-FLU (143)
	(*R*)-2-(3-(4-fluorophenyl)butanoyl)-6-methoxy-9*H*-xanthen-9-one	(*R*)-XEA-4-FLU (144)
	(*S*)-*N*-(1-(3-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S*)-XEA5-3-MET (145)
	(*R*)-*N*-(1-(3-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R*)-XEA5-3-MET (146)
	(*S*)-*N*-(1-(4-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S*)-XEA5-4-MET (147)
	(*R*)-*N*-(1-(4-methoxyphenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R*)-XEA5-4-MET (148)
	(*S*)-*N*-(1-(4-chlorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S*)-XEA5-4-CLO (149)
	(*R*)-*N*-(1-(4-chlorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R*)-XEA5-4-CLO (150)
	(*S*)-*N*-(1-(4-fluorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S*)-XEA5-4-FLU (151)

	(*R*)-*N*-(1-(4-fluorophenyl)ethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R*)-XEA5-4-FLU (152)
	(*S*)-5,7-dimethyl-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide	(*S*)-XEA5 (153)
	(*R*)-5,7-dimethyl-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide	(*R*)-XEA5 (154)
	(*S*)-5,7-dimethyl-9-oxo-*N*-(1-phenylethyl)-9*H*-xanthene-2-carboxamide	(*S*)-XEA5-DES (155)
	(*R*)-5,7-dimethyl-9-oxo-*N*-(1-phenylethyl)-9*H*-xanthene-2-carboxamide	(*R*)-XEA5-DES (156)
	*N*-((1*S*,2*S*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S,S*)-X2ADF5 (157)
	*N*-((1*R*,2*R*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R,R*)-X2ADF5 (158)
	*N*-((1*S*,2*R*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*S,R*)-X2ADF5 (159)

	*N*-((1*R*,2*S*)-2-hydroxy-1,2-diphenylethyl)-5,7-dimethyl-9-oxo-9*H*-xanthene-2-carboxamide	(*R*,*S*)-X2ADF5 (160)